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RADIATION AND AGING

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RADIATION AND AGING

Synopses of Panel Discussions
Held During 1958 and 1959

ORGANIZED BY THE American Institute of Biological Sciences
AT THE REQUEST AND WITH THE SUPPORT OF THE
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EDITED BY Philip Handler

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Introduction

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In the course of studies on the influence of high-energy radiation upon animals, shortly after World War II, several investigators, including Boche, Brues, Casarett, Furth, Henshaw, Landis, and Sacher, observed that irradiated animals which had survived the acute radiation syndrome exhibited a significantly decreased life expectancy. To be sure, the incidence of neoplasia in such animals was significantly increased, as compared with unirradiated controls, but this was insufficient to account for the generally observed decreased life expectancy. Early studies indicated that such animals exhibited a wide variety of causes of death, apparently resulting from diffuse deterioration of tissues. In general, when tumor-bearing animals were discounted, the causes of death in the irradiated animals did not seem to be statistically different from those observed in normal, aging animals. Hence, Blair suggested that radiation occasions "a general acceleration of the normal aging process"; since then the statement has been made, frequently, that the late effects of ionizing radiation resemble the manifestations of normal biologic aging. Indeed, the most superficial look readily reveals striking parallelisms. Life expectancy is decreased in a linear fashion with increasing radiation dose as well as with the increased passage of time. The incidence of leukemia is increased as a linear function of the radiation dose and, in general, there is an increasing incidence of malignancy during normal aging. Irradiated animals exhibit a diminished LD_{50} with respect to subsequent radiation exposure and, in general, the larger the initial dose the lower the LD_{50} for the second exposure. Similarly, with the passage of time, unirradiated animals exhibit a lower tolerance to an acute irradiation dose. Finally, in both instances, there appears to be no specific organ, tissue or physiological function in which a primary defect seems to be lodged; instead, one observes a generalized loss in many, most, or perhaps all tissues.

As a consequence of various discussions, Drs. G. Halsey Hunt and Stanley Mohler, representing the Center for Aging Research of the National Institutes of Health, and Dr. Paul S. Henshaw, representing the Division of Biology and Medicine of the U. S. Atomic Energy Commission, concluded that the question whether ionizing radiation actually advances the degree of biological aging in an individual organism could be clarified significantly if currently existing information were brought to bear. Through a joint effort, arrangements were made with the American Institute of Biological Sciences to conduct a series of panel discussions with the following objectives: *a*) to bring together and organize concepts pertaining to the similarities and dissimilarities between features associated with "normal" biological aging and radiological shortening of life, respectively; and *b*) to identify aspects of the general program which may require further research and for which the time appeared to be propitious.

To meet these objectives, six sets of panel discussions were organized. The panel chairmen were urged to gather an appropriate group of knowledgeable investigators of diverse experience and disciplines to discuss, from their standpoints, the aforementioned common problems. The topics to be discussed and the respective panel chairmen were: *1*) Pathological Aspects, Thomas F. Dougherty, Department of Anatomy, University of Utah; *2*) Biochemical Aspects, Philip Handler, Department of Biochemistry, Duke University School of Medicine; *3*) Physiological Aspects, Laurence W. Kinsell, Institute of Metabolic Research, Oakland, California; *4*) Cytological Aspects, Theodore T. Puck, Department of Biophysics, University of Colorado Medical Center; *5*) Genetic Aspects, John W. Gowen, Department of Genetics, Iowa State College; and *6*) Behavioral Aspects, Ewald W. Busse, Department of Psychiatry, Duke University School of Medicine. Each panel convened during 1958 and 1959; 12 panel meetings were held in all, each involving 8–12 investigators. Nathan W. Shock of the Gerontology Unit, Public Health Service, Baltimore, and Ralph Brauer of the Radiological Defense Laboratories, United States Navy, San Francisco, served as consultants to several of the panel meetings. The reports that follow have been prepared by five of the panel chairmen and represent summaries of the discussions of the respective panels. The proceedings of the panel on Behavioral Aspects will be published separately. A general summary of these discussions was presented at a symposium on aging held by the American Associa-

tion for the Advancement of Science in December, 1959; this report has since been published (Handler, Philip. "Radiation and Aging," In: *Aging*. Washington, D. C.: American Association for the Advancement of Science, 1960, p. 199). An independent analysis of the problem was published by Bernard L. Strehler (*Quarterly Review of Biology* 34: 117, 1959) as well as in the proceedings of the 1957 conference at Gatlinburg and the 1958 symposium on radiobiology at Burlington.

Clearly the task that was posed remains difficult if not impossible. With insufficient understanding of the mechanisms by which either radiation or the passage of time influence the mammalian organism, it is exceedingly unlikely that one can hope definitively to establish whether the delayed effects of radiation are equivalent to, or mimic those, of aging or whether perhaps the entire relationship is fortuitous and based merely upon the crudity of the original parameter—life expectancy.

Each of the panels found it necessary to establish satisfactory working definitions. The following definition is a composite of statements made at the various panel meetings: Aging is the deterioration of a mature organism, resulting from time-dependent, essentially irreversible changes intrinsic in all members of a species such that, with the passage of time, they become increasingly unable to cope with the stresses of the environment, thereby increasing the probability of death. Note that this definition excludes disease per se, while admitting that aging increases susceptibility to disease. One group noted also that aging is considered that situation in which—within a tissue or organ—catabolism becomes dominant over anabolism with a resultant decrease in functional capacity. In his review, Strehler stated that aging may be viewed as "a change in constitution due to an inequality of the rates of appearance and disappearance of specific chemical structures, changes which are not directed toward the production of a more effectively adapted organism." The most generally accepted medical description of the aging rate in any species is provided by the equation originally formulated by Gompertz:

$$N = N_0 e^{\alpha t}$$

The exponential constant in that equation is a genetically inbuilt feature for each biological species, but the physiological basis for this constant is not clear in any species. It seems quite likely that this constant is itself derived from a series of subconstants reflecting independent rates.

All groups agreed that, in considering the effects of aging or radiation, it is imperative that the particular species be at all times borne in mind. Clearly, it is unwise to attempt to carry over into considerations of metazoan life information known only with respect to bacteria, protozoa, or lower animal forms. Similarly, caution is also required in attempting to carry over into discussion of mammalian longevity factors known to be operative with certainty only, for example, at certain stages in the life of insects.

All groups agreed to accept the general description of the aging process which has been provided by the review by Comfort (Comfort, A. *The Biology of Senescence*. New York: Rinehart & Co. 1956) and by the systematic studies of Nathan W. Shock. Generally accepted also was Shock's thesis that the decline in function of many organ systems in the mammal reflects the simple loss of postmitotic cells in nondividing tissues. In general, this is expressed as a decrease in vital capacity, in muscle strength, in renal function, in mental acuity, and in cardiac activity, while, at the same time, there occurs little or no decrease in the activity in bone marrow, intestinal epithelium, or liver—tissues in which mitosis is significant throughout life.

None of the reports that follow are to be considered complete, systematic reviews of the field or even of the particular aspect with which each panel was concerned. Each of them, rather, should be considered as consisting of the highlights of the panel's discussions, colored by the prejudices of the panel chairmen.

Similarities between physiologic and radiologic accelerated aging

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THERE ARE NUMEROUS REPORTS that X-irradiation of animals has a life-shortening effect (1). Other, more indirect data indicate that the expected life span of man may also be shortened by repeated exposures to X-rays (2). It must be emphasized that the life-shortening effect of irradiation would be of merely toxicological interest if it were owing to pathological changes uniquely produced by X-irradiation. However, exposure to small amounts of X-irradiation and possibly to some internally deposited radiosiotopes may shorten the life span by nonspecific mechanisms, i.e., degenerative diseases and accelerated the altered physiological processes similar to those found in not irradiated, aging animals and man. It was to consider this problem that a conference was convened at Brighton, Utah, on September 20, 1958. Visiting participants were Drs. Thomas F. Dougherty (Chairman), Warren Andrew, Henry Blair, George Casarett, Stanley Mohler, Frederick Stone, Harry Sobel, Nathan Shock, Terrence Cochran, and Don Wood. From the Radiobiology Laboratory of the Department of Anatomy, University of Utah, the following staff members attended: Drs. Betsy Stover, Jean Dougherty, Carl Rehfeld, Webster Jee, Glen Taylor and Mr. C. N. Stover. What follows is a summary of the thinking that derived from that conference.

There seem to be two ways by which X-rays (and possibly other types of irradiation) may shorten life: *1*) by increasing the production of certain diseases (e.g., neoplasms) and, in larger doses, by inhibiting adequate repopulation of transient tissues (to an extent greater than in nonirradiated populations); and *2*) by possibly shortening life, by accelerating the rate of appearance of degenerative alterations. Tumorigenic effects pose problems that are related to the etiologic aspects of neoplastic growth, but may not have great bearing on the less directly related effects of X-irradiation on acceleration of specific degenerative changes of aging. Those pathological changes produced by enormous amounts of radiation should be excluded from these two categories, even if they have features common to other illnesses, since the immediate cause of death (acute radiation disease) is due to the radiation per se, and does not depend on a chronic process accompanying aging.

The author is well aware that many investigators include cancer production as a radiation-accelerated life-shortening process, but cancer is not necessarily a degenerative disease, although it shortens life. The real problem is: does radiant energy accelerate the development of those inadequate cellular and tissue functions that are normally associated with the passage of time in populations not exposed to greater than background radiation? These changes also may be related to tumor development, but this problem is so extensive we should consider it separately.

EXPERIMENTS DESIGNED FOR STUDY OF RADIATION-ACCELERATED AGING[1]

It has not been proved that radiation specifically induces any one particular degenerative disease in all species. Control animals (not irradiated) are essential within the species used for experiments and, if possible, with strains within the species. In addition to the genetic background of animals, environmental conditions (including the nutritional environment) should be standardized and controlled for all animals under experimentation. Whenever possible (as in mouse strains) it is also desirable to establish the incidence of different diseases and the alterations in metabolism and hormone balance that occur during aging and that might influence the experimental results among irradiated groups.

All of the foregoing factors are important to evaluate, since the same fundamental change could be induced by irradiation in different species, but the innate capacities of the different species to acquire the same pathological alterations may differ. In other words, the stimuli to accelerated disease incidence may be quantitatively and qualitatively the same and may induce essentially similar immediate responses, but the diseases which develop in different species and even among strains of one species may be dissimilar.

[1] Radiation-accelerated aging is a term used here only to indicate that radiation was employed in experiments in which the animals exhibited premature signs of aging. It does not mean that physiologic (non-radiation induced) aging is identical to radiation effects.

The most fundamental cellular and tissue alterations that irradiation may induce in different species may be the same, but the characteristic features of accelerated aging and the pathological alterations may differ according to the different susceptibilities of the species studied. Thus, irradiation, acting by mechanisms common to all animals, may accelerate not single change, but a wide variety of age-dependent pathological changes that are similar to those expected during the normal aging process and unique to a particular species.

ROLE OF CELLULAR DAMAGE

One of the most fundamental problems both in the aging process and in radiation-induced changes is the relation of cellular damage to ensuing events. Damage to cells having different degrees of radiosensitivity results in different pathological alterations. Also, radiation may damage cells not only directly but also by indirect means. Thus, destruction of the most susceptible cells (or the cells hit) may, in turn, trigger waves of cellular destruction (among cells not hit) through cytotoxin elaboration and thus result secondarily in a series of pathological alterations not immediately connected with the original cells destroyed. For instance, cells of the crypts of Lieberkühn are highly susceptible to irradiation; once many of these cells have been destroyed, the secretions may be so reduced that there is a lack of adequate enzyme-production, which, in turn, would interfere with digestion in the duodenum and lead to a series of secondary effects. Thus, damage to a particular type of cell, with the ensuing absence of its normal function, may lead to a series of events apparently unrelated to the initial effects of irradiation.

Another example of an indirect effect of irradiation is a release of toxic substances when cellular damage occur. By "toxic substances" we mean those substances that are released by damaged cells and that cause destruction of other cells—cells which may not have been irradiated. Histamine and 5-hydroxytryptamine are examples of such substances. Any stimulus, including X-irradiation, that will produce cellular damage can bring about the release of these substances. Cytotoxins, once released, can set off a series of phenomena as a consequence of their action. In general, then, a large variety of stressful circumstances, including irradiation, which cause cellular damage and cytotoxin release through both direct and indirect effects, could induce changes common to all. In this way, aging processes that result from recurrent cellular damage and repair could be enhanced in essentially the same fashion by a group of stressors, among which radiation is not unique.

CELLULAR REPOPULATION

After cellular damage has been produced and the cellular debris removed, there is a phase during which repopulation of cells takes place. The degree to which repopulation can occur may, in the case of irradiation, be dose-dependent (number of cells lost) and is also, very likely, age-dependent (number of cells in reserve).

Any situation that brings about a requirement for regeneration of cells multiplies the opportunities for mutations, which have previously been produced, to find expression. The production of mutations and their import has been discussed by another committee, but it seems worth pointing out that such changes may result in inadequate cellular function as well as tumorigenesis. Cells may regenerate, but inadequately, both numerically and functionally, so that the reconstituted tissue may not, for example, be adequately secretory, which it had been before destruction and reconstitution. Cellular loss and regeneration are, of course, a consequence of the vicissitudes of normal aging. In either case, whether during aging or after damage by irradiation, reconstitution of function may be diminished each time some cellular damage occurs. Ultimately, the reconstituted tissue or organ may be unable to maintain adequately its role in the bodily economy.

Whenever repeated damage and reconstitution occur, a series of pathological changes, which are only indirectly related to the cause of the original cellular damage, could follow. For example, anything that might, over a period, tend to bring about a diminution in the number of β cells in the islets of Langerhans could, indirectly, bring about the earlier development of arteriosclerosis. Successive periods of requirements for reconstitution of transient cell populations could lead by direct and indirect means to an inadequacy of organ and tissue reserve. Such loss occurs during physiologic aging and could be markedly accelerated by irradiation.

One point that provoked some discussion is the concept that the decrease in organ reserve during aging or after irradiation may not be owing only to an inadequate or improper function of the cells composing the organ, but mainly to a decrease in the total number of cells. For example, in animals injected with radium or plutonium in doses causing chronic changes, it appears that a loss in bone-marrow mass (3) may be a major factor in accounting for the early anemia which reflects production of insufficient numbers of essentially normal erythrocytes. In other words, the total factory for the production of red corpuscles and, as a result, its secretions (holocrine) may be diminished rather than the remaining marrow having improper or inadequate functioning. The irradiated animal may function adequately under optimal environmental conditions but may fail to adjust to increased requirements of additional stress. With illness (e.g., intercurrent infections) the marrow may be pushed to produce more corpuscles, at which time the lack of functional reserve may be revealed. So far, no alteration in the rate of production or in the life span of the red cells has been demonstrated in these animals (4).

Among progenitive cells, proliferation is of two general types: *a*) the maintenance of a seed bed, i.e., the germinal element, and *b*) furnishing cells that go through maturation processes to form an end product. During aging and after irradiation, there may be a diminution in the

number of cells retained for generative purposes. Thus, the balance between generative production and maturation may shift with time. However, the reduction in capacity to form the end product of maturation may be owing to a total reduction of cell mass, and the ratio of cells undergoing these two processes within any particular progenitive tissue may remain the same. For example, if the requirement for hemoglobin should increase, then it is possible that the factory, so to speak, would not be able to meet the demand and, in trying to do so, would reveal its inadequacy.

In summary, it may be that the margin of organ or tissue reserve is smaller in older individuals and possibly also in chronically irradiated animals. In the latter, functional requirements may be increased by the stress produced by irradiation; at the same time, the capacity to replace cells in irradiated organisms may be diminished, and the aging process is enhanced.

The question was raised concerning the advisability of separating the reduction in functional reserve of a tissue from the loss of capacity to recover. It was felt that there should be a separation between these two phenomena. It should, however, be kept in mind that—if there is truly a reduction in numbers of progenitive cells as an animal ages or after irradiation—regardless of whether immature cells recover from various injurious agents, the total number of these cells of older animals would still not be as great as in the younger animal.

Among progenitive tissues or organs, the skin also would be expected to react in a similar fashion to bone marrow. During aging, there is a thinning of the skin and a decreased number of papillae, with the concomitant apparent decrease in capacity to replace the outer layers of epidermis. Doses of irradiation required to eliminate ridges in the skin are very high. Apparently, the amount of irradiation required to produce acutely changes that are similar to those of aging are very high. However, we know little about whether or not small amounts may accelerate aging. A most interesting question of considerable importance was raised concerning what would happen if old marrow is reseeded with young marrow cells or young skin is transplanted into an area of old skin. This type of experiment would yield data concerning the role of the internal environment of an old animal on young cells, and vice versa.

The hypothesis that there is a decline of the total number of progenitive cells could be tested for hemoglobin production by ascertaining the recovery of the rate of iron incorporation after two doses of X-rays, spaced considerable periods apart. There is relatively little data, if any, on experiments of this type that have already been done. With continuous exposure, the red-cell population declines, establishes a new equilibrium, and stays at this level for a long period. This is apparently true not only of chronic exposure to low levels of X-rays, but also to low levels of internal emitters, e.g., radium. This fact can be taken to imply that there is a loss of recoverability. On the other hand, it is possible—and we believe there is some evidence for this point of view—that basically there is not an impairment of synthesis of hemoglobin but that there is actually a gradual decrease in the total amount of marrow progenitive cells.

Vascular and parenchymatous aging. Dr. Casarett presented data on the histopathological changes in the arteriocapillary beds (5, 6). Radiation induces an alteration in the small vasculature and surrounding connective tissue that precedes the onset of specific diseases in locally irradiated areas as well as in the totally irradiated animal. It was pointed out that, from this standpoint, localized premature aging is a real entity. The localized process results in irreversible radiation injury, and is comparable to a fundamental, not-radiation-induced change found during aging, i.e., damage to the vasculature and the accelerated senescence of parenchymal tissues. Similar changes in the fine vasculature of bone after the administration of bone-seeking isotopes were also described by Jee and Arnold (7). The disturbed vasculature was occluded and resulted in osteocyte death. This was shown by India-ink and gelatin injections. Several different alterations in cellular activity follow the reduction in vascularity. For example, there is an inadequate degree of repopulation, altered regeneration, and possible incapacity for regeneration to occur. In addition to the reduced capacity of parenchymatous cells to function, the details of the changes described by Casarett occur in several phases, and their extent is dose-dependent.

In general, low doses of irradiation may accelerate changes that otherwise occur during normal aging (6). Changes similar to those in the capillaries have also been described after anaphylactic shock and other stressful circumstances, such as the administration of histamine and other toxins (reviewed by Dougherty (8)). Thus, the changes described by Casarett may not necessarily be solely owing to radiation, but to an altered capacity of endothelial cells and other connective-tissue elements to maintain themselves in optimal functioning state following exposure to a wide variety of stressors.

The first alteration seen microscopically after irradiation is spotty degeneration of endothelial cells. There is swelling of the endothelium before the final destruction of the cell occurs, and this is accompanied by a movement of surrounding fibroblastic cells to the area of necrosis. This fibrotic change is so gradual and so minute that at any one time large numbers of altered capillaries and arterioles may not be evident unless, of course, severe stress or larger doses of irradiation are given. This pathological change also may be considered to be another evidence of a chain reaction set off by initial cellular damage; that is, when a few cells may be damaged they, in turn, may bring about—through release of their substances—a series of changes in surrounding tissue, so that it is not necessary to postulate that every single endothelial cell damaged may be the object of a direct hit.

The loss of arterioles and the ensuing fibrosis occur as a rather evident process in certain organs that undergo early degeneration, e.g., the thymus, the pineal body, and the vermiform appendix. Similar changes may be

observed during fibrosis of the thyroid. These changes may occur at different times in different organs during the normal aging process, and be so gradual that they are very difficult to quantify. One way of quantifying such alterations is to induce arteriolocapillary fibrosis, and then actually count the capillaries per unit volume of tissue. This would be laborious, and it would be worthwhile to have some way of approaching the problem in a more indirect, but measurable way.

If such changes in vascularity after irradiation were generally distributed throughout the body, numerous alterations in parenchymatous-cell function would follow the consequent reduction in the blood supply. As has been pointed out elsewhere, changes in the function of the central nervous system could be a consequence of such altered vascularity. It has been mentioned with respect to bone (7) as well, that the loss of blood supply also could account for various bone diseases, which are not necessarily sarcomas, but are owing to the altered architecture of the bone itself.

The question was raised as to whether there was any information concerning the ability of animals that have lost much of their vasculature by this mechanism to sustain partial anoxemia. The members of the committee were not acquainted with such studies, if, indeed any have been made. An obvious consequence of the theory presented by Casarett is that the accelerated aging produced by a given amount of radiation would depend on the amount of previous age-change already present. If such vascular changes were proceeding at a certain rate in a given population, held under constant environmental conditions, then irradiation given to such animals would enhance the rate at which these changes occur, as compared to the nonirradiated population. Termination of life in individuals of this population would depend on the changes that had already taken place in certain organs and tissues. Casarett (6) particularly pointed out that attention should be paid to the kidney, in which small vascular changes can bring about distant but related pathological alterations through accelerated vascular changes, such as arteriolosclerosis, hypertension, and so forth, which, in turn, bring about pathological conditions in other organs. So, it may be seen that once an animal is subjected to irradiation, its life span could be shortened through a series of vascular changes which would not, apparently, be related to the direct effect of the initiating stimulus.

Vascular plugging. In addition to the change in the endothelial cells and the multiple fibroses that occurs in the injured capillaries or around the capillaries, there is another phenomenon, induced by radiation, which also brings about a diminution in the blood supply. The presence in the blood of a mucopolysaccharide that plugs small capillaries and even arterioles has been noted by several different workers. More recently A. C. Anderson (9) has considered this problem in detail. The substance is PAS-positive; it is found in small blood vessels after relatively large doses of X-irradiation. The source of this polysaccharide protein complex is not known. Similar material has been found in small vessels in the haversian systems of bones of animals subjected to internal irradiation from bone-seeking isotopes (7). This work tends to show that the loss of vascularity in bone is related, at least in part, to the vascular plugging effect of what is presumed to be a substance similar to that described by Anderson. Jee and Arnold (7) also reported on the fact that there is diminution in vascularity in old bone not subjected to irradiation. Whether the two processes are the same is not certain at this time; the end result, however, is similar.

Connective-tissue changes found during aging and after irradiation were discussed at some length. This subject, like tumorigenesis, is so extensive that it must be left for a subsequent, detailed report. The effect of radiation on ground substance, the possible depolymerizing effect on this mucopolysaccharide, and how this is related to aging were discussed. The question of application of hexosamine collagen ratios to the study of normal aging and radiologically induced aging tissues was explored, but time was insufficient to go into this matter as thoroughly as it is hoped might be done in the future.

ALTERATIONS IN BLOOD VOLUME AFTER RADIATION AND AGING

If there is a decrease in the vascular space during aging and after radiation, one might obtain some evidence for this change from studies of the total blood volume. Evidence has been presented (10) that, in dogs made anemic by injection of various bone-seeking internal emitters, there is a relatively greater decrease in the body hematocrit than in the venous hematocrit. This means, of course, that a significant volume must lie outside the vascular bed. In discussing the fact that plasma leakage occurs after irradiation, Brauer noted that it is difficult to account for the large amount of plasma present in the liver on the basis of hemoglobin content. In any case, the data tend to indicate that there is a real reduction in the amount of hemoglobin, regardless of the mystery of where the plasma is. Since the venous hematocrit remains approximately normal and there is a marked reduction in the total amount of hemoglobin, a significant reduction in the vascular space is the only obvious explanation of this finding.

Further studies should be made to determine the rate of this reduction in total hemoglobin during normal aging and in radiologically induced aging. As the vascular bed and the blood volume diminish during aging and during radiation-induced aging, additional stress (i.e., greater than that encountered in an optimal environment) may tax an individual beyond his capacity.

In a summary of the discussion of the changes in the vascular bed and the accommodation of the blood volume to the vascular space, it was suggested that radiation is not the only way in which such changes could occur; i.e., that multiple stresses during life might produce similar effects, mediated through the release of

substances from dead and dying cells which induce degeneration in other endothelial cells, thereby bringing about the progressive diminution. There is an additional component in degeneration, since some stressful circumstances tend to increase the vascular bed; e.g., exercise may enhance muscle strength. In addition to stress responses, there must be a time-dependent, irreversible, degenerative feature. The loss of vascular space offers this feature, since it limits the capacity to repair and regenerate.

BIOLOGICAL TIME CLOCKS

Quantitative changes which occur in specific organ systems were examined for time-dependence. Lymphatic tissue changes with time in a definite sequential fashion (11). There is a rapid, prepubertal growth; this is followed by a decline that is most rapid just after puberty and then tapers off, but continues at a rather steady rate during the rest of the life span. This is the only tissue in the body which behaves in this fashion. Numerous studies have been made of this chronological event, and it has been shown to occur regardless of body weight, so that there is a continuing decline in the amount of lymphatic tissue in the organism with time. Although hormones can markedly influence the amount of lymphatic tissue present at any age, the rate of decrease is unmodified by known hormonal actions.

Lymphatic tissue is exceedingly sensitive to both external and internal irradiation (11), the effects being similar to those of the steroid hormones of the adrenal cortex (11). Both radiation and the adrenocortical hormones exert destructive effects on lymphocytes, and this is followed by a rapid wave of reconstruction to an amount of tissue that is characteristic for the age of the animal. Thus, there is a lymphatic tissue time clock. Much is known about the age-involution of the thymus, yet little attention has been paid to the behavior of lymphatic tissue, in recent years, by those interested in the aging process. The importance of the age-involution of lymphatic tissue is not known but it could be related to the function of this tissue. If lymphatic tissue produces certain serum proteins (β and α globulins), then the less tissue available to do so, the smaller the reserve capacity for synthesis of these proteins as aging progresses. Both X-irradiation and adrenocortical hormones will inhibit antibody (γ globulin) synthesis. Also, lymphatic tissue may yield nucleic acid or its derivatives, which may be of importance to the functioning of other cells.

Radiation reduces the amount of lymphatic tissue by depleting it of lymphocytes. By adrenocortical mediation, stress has a similar effect, and the aging process is accompanied by a progressive loss of lymphatic tissue. All these processes reduce the amount of potential function of lymphatic tissue, which may be of significance for the adequate maintenance of other cellular functions.

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Biochemical considerations of relationships between effects of time and of radiation on living systems

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THE PANEL which was convened to consider biochemical aspects of the possible relationship between the biological effects of radiation and the normal process of senescence consisted of the following group of investigators of diverse backgrounds and research interests: as radiobiologists, Henry I. Kohn, Harvey M. Pratt, and George Sacher; Nathan W. Shock provided his broad knowledge of the field of human aging; Jerome Gross, Harry Sobel and Marott Sinex represented the large group of investigators currently considering the behavior of connective tissue in aging animals and man; Donald Pickering and Urner Liddel provided the point of view of the biophysicists; the biochemists were Philip P. Cohen, Emil L. Smith, William D. McElroy, and Philip Handler. Ralph Brauer combined his experience as a radiobiologist and a student of the liver and introduced the known parallels between the effects of radiation and the behavior of normal, aging, nonirradiated animals.

It early became clear that all panel members, as an article of faith, subscribed to the concept that a true analysis of the problem at hand was not possible and that it would not become possible until the effects of radiation and the nature of the aging process had been described in terms of chemical changes in the structure or behavior of cells or their environment. Since, in point of fact, almost no such information is available, the group was forced to a comparison of certain parallels in the known behavior of radiated and aging animals and to speculate upon conceivable operative mechanisms. What follows, therefore, is a summary of the concepts considered to be important and relevant to the problem at hand.

Physical parallel. Since irradiated animals, in general, die sooner than do their nonirradiated controls and since, except for neoplastic lesions, the causes of death are distributed in both groups in a roughly equivalent manner, it has been stated that radiation "accelerates" aging. There appear to be three components to this statement that are independently subject to analysis. There can be no denying the fact that irradiated animals die sooner than do the nonirradiated controls and that the differential is a function of the radiation dosage. There appears to be inadequate documentation of the notion that the causes of death are distributed approximately equivalently in both groups and the panel awaits a reasonably large scale systematic comparison of this nature. Should the generalization fail, then a clue will have been provided concerning the more specific nature of the delayed effects of radiation. An eloquent plea for such studies and a related blueprint for a concerted attack upon the nature of radiation effects has been described by Henry Kohn in the Proceedings of the 1958 Burlington Conference on Radiation and need not be reviewed here. Finally it must be noted that the term "accleration" is misapplied in this context since, if the term were used in its usual physical sense, it would imply that the influence of radiation on the mortality rate should increase with the passage of time. Instead, however, Sacher's analysis of the response to varying radiation dosage indicates that, whatever the basis for this phenomenon, it happens within a relatively brief period after exposure. This is indicated by the fact that, immediately after irradiation, the mortality statistics for the irradiated population resemble those of a significantly chronologically older population. Were the term acceleration correct, this would be indicated by an increase in the exponential constant in the Gompertz equation. Instead, examination of the mortality curves for normal and postirradiated animals reveals that the curve for the irradiated animals closely resembles that of their nonirradiated controls but displaced forward in time. That is to say, it would appear that the irradiated animal has aged months or years within a few minutes after irradiation. Thus, as a first approximation, the mortality figures obtained after exposing a colony of 2-month-old rats to a single dose of 400 r resemble those obtained by following the behavior of a colony of approximately 1-year-old rats from that day forward. This concept will have become much more meaningful when there has been gathered a larger body of data describing the fate of animals subjected to fractionated doses at varying periods during the life span. Even such experiments must be judged with caution since some phenomena associated with normal aging do accelerate with time, e.g., the incidence of neoplasia and of amyloidosis.

The panel found itself in accord with Sacher's thesis that the statistics of mortality following irradiation are

compatible with the concept that the prime phenomenon responsible for life shortening in such animals is damage to cell nuclei. Since nondividing cells successfully withstand relatively enormous doses of radiation, it would then be expected that the influence of radiation would most readily be detected in the constantly proliferating tissues, e.g., bone marrow, skin, intestinal epithelium, etc. Were this the case, then an immediate contrast between the delayed effects of radiation and normal senescence would become apparent since in the latter it is the nondividing tissues such as brain, myocardium, muscle, kidney, etc., which exhibit the classical decline in physiological function with time whereas the proliferating tissues flourish even in centenarians. The evidence which clearly implicates alterations in the cell nucleus as responsible for subsequent effects of radiation is summarized by Puck elsewhere in these reports.

Chromosomal damage in the postirradiated animal. The concept that, like the *acute* effects of radiation, long term effects might also be the result of chromosomal damage would be much fortified if there were tangible evidence of such damage. Such evidence is provided by Brauer's observation that, in rats subjected to partial hepatectomy as long as 6 months after exposure to 200 r of whole body radiation, whereas regeneration appeared to be effective when judged by the rate of increment of new tissue mass, nevertheless, as many as 50% of all such regenerating cells exhibited abnormal mitotic figures. No estimate is possible of the extent of reproductive death in these regenerating livers; only those cells wherein the damage was insufficient to result in reproductive death were visible to be counted under the conditions of the experiment. It must be emphasized that despite the extremely high incidence of chromosomal aberration in these regenerating livers, there was no evidence of inadequacy of hepatic function. It is conceivable that each of the cells which showed visible chromosomal damage also was affected in such a way that one or more of its normal metabolic potentials was impaired. However, since in the regenerating liver, cells are not cloned and on the assumption that there are no specifically highly favored hot spots in the chromosomal gene maps of liver, then, presumably, the metabolic deficiencies of any one cell would be compensated by the activities of the surrounding tissue.

Similar chromosomal damage in the nondividing cell lines of the mammal would not be evident by ordinary cytological examination. The question then arises as to whether such occult damage could abbreviate the life of the cell in which it had occurred. Clearly no definitive statement can be made in this regard. The role of the nucleus in a mature differentiated cell is as yet uncertain. It is generally acknowledged that the concept of turnover of the constitutive proteins of mammalian cells was somewhat exaggerated in its original formulation. There is a serious need for re-examination of this question in precise quantitative terms. However, since it is clear that some protein is made at all times even in mature cells there is a constant need for "instructions" with respect to the mode of their synthesis. Available evidence indicates that such instructions are provided by the ribosomal nucleoprotein, seemingly under the influence of "messenger RNA." If the latter, as appears likely, is provided by the nucleus, receiving its instructions from the nuclear DNA, then the necessity for an undamaged nuclear DNA throughout life will be obvious. But since mature mammalian cells are at least diploid and frequently polyploid, statistically it seems most unlikely that in any cell all the genes bearing information for the synthesis of a specific protein will have been damaged by radiation. Accordingly, at most it would seem that such a cell will have been converted by radiation to the heterozygous state and hence retain the capacity for synthesis of its total protein complement. The only exception, of course, would be those diploid cells which were already heterozygous and in which the normal genes were then destroyed by the radiation. The frequency of this phenomenon, however, would seem to be relatively insignificant and unlikely to account for a significant degree of cell death in the postirradiated animal.

Nevertheless the panel felt that it was quite conceivable that such chromosomal damage with its potential failure of protein synthesis could conceivably account for eventual cell death or failure of one of its functions.

It will also be clear that if radiation damage is lodged in the chromosomes of a young animal, at a time when cell division is still prominent in all tissues, then optimal conditions will have been created for somatic expression of the genetic damage and the incidence and frequency of cell death on this basis would be markedly enhanced. Conceivably, it is for this reason that prevention of the growth of young irradiated animals by the McCay dietary regimen in which growth is prevented by caloric restriction, markedly extends the life time of such irradiated animals. It is particularly noteworthy that, when such animals are realimented with a normal diet, the normal extremely rapid growth response is not seen but the incidence of mortality rises sharply.

FUNDAMENTAL ASPECTS OF AGING

The panel agreed that the data of Nathan Shock clearly indicated that no significant differences between the metabolic functioning of aged and young tissues were apparent when such parameters as oxygen consumption, tissue enzyme activity or the circulating level of various metabolites in peripheral blood are considered. Perhaps the single generalization which appears in such data is that aged animals invariably exhibit a relatively sluggish or delayed response to physical or metabolic stresses such as insulin administration, cold, etc. The panel also concurred with Dr. Shock's conclusion that the most rational single explanation of the functional impairment of the aging tissue is that it reflects an absolute loss in the cell population with replacement of these cells by extracellular fluid or connective tissue—the "cell deletion" hypothesis. Having accepted this suggestion it appeared profitable to consider possible mechanisms underlying such cell deletion.

An attractive possibility is the progressive loss of cells due to "genetic death" resulting either from a hereditary genetic defect or in consequence of radiation. This hypothesis is attractive since it would serve to unify concepts of both the aging process and of the late effects of radiation. Unfortunately, it is extremely difficult if not impossible to obtain definitive indications of this kind of phenomena. Clearly, if aging is a consequence of progressive cell death with deletion, this is an extremely slow process in mammals. Since, by available parameters, tissue specimens removed from aged animals appear to function normally albeit perhaps somewhat more slowly than do tissues from young animals, the number of "sick" cells in the total cell population at any time must be vanishingly small. This would be equally apparent when considering the difficulties inherent in demonstrating specific changes in a cell population irradiated *in vivo*. As indicated earlier, even if irradiated cells were to sustain damage to their genetic apparatus, this would be difficult of demonstration since it is quite likely that in each cell a different mutation may occur so that analysis of the total tissue for the activity of various enzymes or for the presence of various proteins would show no significant aberration from the norm. Of interest in this connection is the demonstration by Pratt that if partial hepatectomy is repeated several times in young and in old mice, regeneration is slower and less complete in the latter group. This is also apparent when the same experiment is performed on normally aging and irradiated mice of equivalent age; again, the livers of the irradiated animals show somewhat less complete and slower regeneration. The data of Brauer, referred to earlier, clearly indicate that in the irradiated animals this might well be a consequence of genetic damage. But it remains to be established what mechanisms are operative in impairing regeneration in the livers of old, nonirradiated animals.

Thus, while the panel was much attracted to the hypothesis that the premature death of cells in postirradiated animals may be a consequence of genetic cell deletion, it was felt that the evidence for the concept was at best suggestive. Further, there appears to be no categorical evidence that normal aging is necessarily an analogous process although the possibility has by no means been eliminated.

It appeared fruitful therefore to consider other possible mechanisms which might account for progressive, time-dependent cell deletion in the aging animal. Of those considered, most attractive was the possibility that cell death may be a consequence of failure of communication of the cell with its environment, i.e., the result of an inadequate supply of metabolites or removal of metabolic products. Restated, this hypothesis suggests that normal aging may be the penalty which a complex organism pays for its multicellularity. As contrasted with unicellular forms of life which may, potentially at least, be immortal, the multicellular animal is distinguished by the fact that its cells are separated from their food supply and from the atmosphere by connective tissue. The quantitative significance of connective tissue will be appreciated from the fact that its characteristic protein, collagen, is 40% of the total body protein of the rat and is as much as 85% of the total protein of the sponge. Two other fibrous proteins are found in lesser amounts in connective tissue, viz, elastin, and reticulin. Elastin, like collagen, contains 30% glycine, but, unlike collagen, contains no hydroxyproline or hydroxylysine. The cellular origins of elastin and reticulin are not certain. Occasional suggestions in the histological literature that elastin is derived from collagen seem most unlikely when their chemical structures are considered. Indeed when such a transformation seems to have occurred in any tissue, from a chemical standpoint this would seem possible only if the collagen had been removed and replaced by entirely new molecules of elastin. Reticulin appears as thin, branched, silver staining fibers in young tissue, but seems to disappear with time as the animal matures and is replaced by the thicker collagen fibers. Conceivably the reticulin does not really disappear but simply cannot be readily observed in the presence of the much more abundant collagen.

The other typical component of connective tissue is the ground substance, an amorphous organization which has been insufficiently characterized and is presently known only to be composed of various mucopolysaccharides such as the chondroitin sulfates, hyaluronic acid, keratosulfate, etc.

The possible role of connective tissue in the aging process may be summarized as follows: at the capillary level, connective tissue affords a selective diffusion barrier which must be crossed by all food stuffs and metabolic products. Should the properties of this diffusion barrier alter with time in such manner as to interfere with the ready passage of metabolites, conceivably it might also result in failure of cellular function with subsequent cell death. Thus, aging would again be attributed to cell deletion, but the cellular death would result not from inherent or induced genetic defects of somatic cells but to their inadequate nutrition. Unfortunately, no experimental means have been devised to put this hypothesis to definitive tests. Rather, various groups of investigators have been examining the behavior of both the protein and the mucopolysaccharide components of connective tissue as they exist in aggregates which make experimental observation possible, e.g., the behavior of such structures as tendons, skin, and intervertebral discs.

Gross has demonstrated that when the skin of young animals is extracted with saline solutions, it readily yields a mixture of the materials of the ground substance and a gel of collagen. However, if similar extracts are made from skin of older animals, the amount of extractable material decreases markedly and little or no collagen is obtained in the extract, despite the fact that both by light and by electron microscopy it is apparent that old skin contains more, rather than less, collagen. This behavior can be mimicked by the behavior of collagen preparations *in vitro*: a cold, sodium chloride

solution of collagen is crystal clear. If warmed to 37° the solution sets to a gel in which can be found typical collagen fibers when examined by electron microscopy. If the gel is cooled, the gel fibers return to solution. However, if the gel is maintained for some time at 30°, the ability to redissolve on cooling disappears, thus suggesting that with time there is increasing cross linking between the collagen fibers which become more "crystalline" and hence less soluble. The decreased extractability of skin collagen with the age of the animal may well reflect a similar process. These observations are compatible with the fact that collagen, an extracellular protein, does not participate in the more general turnover of body proteins. Once laid down in the connective tissue, collagen molecules survive for almost the entire life of the animal. Unfortunately no parallel data are available to describe the organized structure of the ground substance of parenchymatous organs or of capillaries. Clearly, however, were similar changes to occur in the organization of the protein of the capillary structures, in time such a process might well interfere with the nutrition of the surrounding cells.

There is no doubt that the organization of the mucopolysaccharides of the cornea, ciliary body and choroid plexus is of considerable importance in regulating the composition of the aqueous humor and of cerebrospinal fluid and, in all likelihood, are of great significance in capillaries elsewhere as well as in the fluid intercommunication between the cells of parenchymatous tissues. The only indication that radiation may induce changes in the organization of the connective tissue has been the finding that the ratio of hexosamine-containing polysaccharide to collagen falls significantly in postirradiated animals with an actual increase in collagen and a decrease in the mucopolysaccharide component. Conceivably this may not reflect a direct effect of the incident radiation on the cells responsible for the metabolism of connective tissue, but rather, may be the result of increased adrenocortical steroid secretion in such animals. But whatever the immediate mechanism, by this indirect means the postirradiated animals might exhibit a hastening of the normal changes in connective tissue with time.

Time-dependent changes in collagen have been observed in several laboratories which have been concerned with the contracture of tail-tendon taken from young as compared with aged animals. At elevated temperatures, tail-tendon taken from aged animals shows a vigorous and rapid contracture whereas under the same conditions tendon from young animals may not exhibit any contracture but rather may dissolve into the surrounding medium. The molecular basis for this contracture remains somewhat obscure but may be assumed to reflect increasing cross-linkage between collagen molecules with time in the organized structure of the tendon. Whether similar cross-linkages occur between the collagen molecules of less organized structures such as those of the capillary or the interstitial spaces is entirely conjectural.

There is little evidence that radiation can exert similar effects on the behavior of collagen. Direct radiation of collagen preparations even at relatively enormous dosage does not result in an analogous increase in cross-linking of existing molecules but changes have been observed in the composition of the connective tissue of irradiated animals. For example, the testis of the irradiated mouse exhibits a very abrupt weight loss due to the death of the proliferating tissue with relatively little effect on the interstitial tissue. Thereafter the weight of the organ increases due in some part to regeneration of proliferating tissue but in larger measure to sclerosis of the blood vessels. The failure of the testis to regenerate completely and normally appears to reflect impaired nutrition of this organ due to the effect on the blood vessels which are thickened and contain an increase in both the protein and mucopolysaccharide components.

As yet no generalized picture has emerged of the behavior of the mucopolysaccharide components of connective tissue in the course of normal senescence. The most ambitious and comprehensive analysis of this problem of which the panel is aware has been undertaken by E. A. Davidson, who has chosen as his test object the nucleus pulposus, the intervertebral disc of rabbits.

Recent studies in several laboratories most notably those of E. A. Davidson and of Karl Meyer have established several, time-dependent, unidirectional alterations in the composition of the connective tissue of the nucleus pulposus and cartilage. In contrast, the mucopolysaccharides of skin and aorta in the same animal seem to be relatively unaltered with the passage of time. According to Davidson the chondroitin sulfate C and keratosulfate components of nucleus pulposus change in ratio and absolute content throughout the lifetime of several animal species (guinea pig, dog, rabbit, man). The absolute amount of keratosulfate increases, while that of the chondroitin sulfate C decreases, yielding a pattern which throughout the life span is characteristic of the age of the animal. Herniated human discs exhibit a "compositional age," obtained by extrapolation, of between 200–300 years, with virtually the entire chondroitin sulfate C component having disappeared.

Of interest is the fact that alteration of the composition and metabolic activity of the nucleus pulposus may be achieved by several external stimuli: Thus, treatment of 12–15 month-old-rabbits with small doses of growth hormone causes a significant fall in the keratosulfate content and a concomitant rise in the chondroitin sulfate C content: the resulting pattern is characteristic of an untreated animal of about 4 months of age. There is no significant accompanying change in the total amount of identifiable mucopolysaccharide in the tissue. Similar alterations in composition may be achieved by treatment of male animals with androgens or females with estrogens. In each case, however, the mechanism of alteration is different. For growth hormone treated animals, the synthesis of polymer from simple carbohydrate precursors and its resultant turnover are similar to that found in animals less than 3 months of age. Androgen-treated

animals display no measurable turnover of chondroitin sulfate C ($T_{\frac{1}{2}}$ greater than 120 days where for normal animals this is 3–7 days, and after growth hormone 27–30 days) but continuing turnover of keratosulfate ($T_{\frac{1}{2}}$ 25–28 days, normal animals greater than 60 days, after growth hormone 8–12 days). Estrogen treated animals display active turnover of both chondroitin sulfate C ($T_{\frac{1}{2}}$ 18–24 days) and keratosulfate ($T_{\frac{1}{2}}$ 25–30 days). Thyrotropic hormone and cortisone cause no alteration in composition or turnover rates.

The panel also gave consideration to the possibility that there may be one or more built-in, time-dependent, self-destructive aspects of cellular existence, which may in part account for the grosser aspects of senescence. The most obvious possibility is that, with time, genetic defects induced by background radiation may accumulate in sufficient number to occasion cell death. In general, the panel was inclined to discard this concept both for lack of evidence and because it becomes impossible to understand the wide variation observed in the exponential constant in the Gompertz equation for animals of diverse species all of which are exposed to equivalent background radiation. The second possibility was that even within nondividing cells there may be occasional accidents of metabolic existence which result in damage to the chromosomal structure equivalent to radiation effects. The frequency of such accidents might be expected to correlate with the metabolic activity of the cell. However, no such correlation is observable in the mammal and this hypothesis was considered to be relatively unattractive. The panel felt that serious attention should be paid to the possible consequences of a newly emerging metabolic generality. It has become increasingly evident that whereas the metabolic activities of cells reflect the operation of a multitude of enzymes, most of which can be demonstrated to catalyze reversible reactions, metabolic pathways are rarely, if ever, reversible. Thus if one considers a metabolic sequence whereby compound A is transformed through a series of intermediates to compound X, if the cell also possesses means for reconverting X to A, it is unlikely that the same series of enzymes will accomplish the reverse transformation and a quite different pathway must be operative. This has now become clear for most of the major metabolic pathways of the cell. Because of this circumstance, a means is afforded whereby independent control may be exerted over the synthetic and degradative pathways and the advantages to the cell are clearly apparent. By the same token, however, the possibility arises that with only slight differences in the rates of the synthetic and degradative pathways, ultimately there may accumulate within the cell large amounts of a material which is in some manner injurious. This possibility has recently been considered by Strehler, but no actual specific examples are currently available.

Only in recent times have the various feedback mechanisms for the regulation of metabolic events been revealed. Three regulatory devices have now been established. The first, which became apparent in the regulation of energy yielding reaction sequences, is the necessity, in a tightly coupled system for the presence of orthophosphate and ADP. In the absence of this pair, the entire sequence of glycolytic reactions and the citric acid cycle must come to a halt. However, it seems unlikely that this phenomenon is disturbed with the passage of time. The second regulatory device is that in which the product of an enzymatic reaction sequence inhibits the enzyme responsible for the first "committed" step in that sequence. Again, since this is an inherent property of the structure of the enzyme protein it seems unlikely that this would alter with time; however, the third regulatory mechanism may be subject to external or internal influences. This is the device, which has been best demonstrated in bacteria, wherein the presence of the product of the reaction sequence inhibits the very formation of the enzyme or group of enzymes responsible for catalysis of the reactions leading to formation of that product. Little is known of the mechanism whereby such repression is accomplished. Indeed the very opposite phenomenon "un-repression" appears to be a logical mechanism for the formation of differentiated cells from an undifferentiated but totipotent cellular precursor. Clearly, should this mechanism fail with time there would be disastrous consequences for the life of the cell. The analogous process, but opposite in direction, is the operation of inductive devices whereby the presence of substrate results in an elevation in the intracellular concentration of an enzyme which catalyzes some chemical reaction in which the substrate participates. Several such inductive phenomena have been demonstrated in mammalian tissues. Again the mechanism of such induction is unclear but should it fail to operate, results may well be calamitous for the cell.

Finally it appears to be worth considering whether the emerging relationship between subcellular structure and metabolic function may be altered in a time-dependent manner. It is not yet possible to elaborate a complete chemical map of the cell with a definitive list of those enzymatic processes which are accomplished in the mitochondria, ribosomes, other microsomes, cytoplasm, nucleus nucleolus, and other membranous structures. Yet it is increasingly clear that the metabolic existence of the cell is dependent upon the integrity of these structures. Should this integrity be impaired with time there might well be lethal consequences. An example of such a possibility is afforded by the presence in mammalian cells of what have been termed lysosomes, microsomes in which are present, perhaps in a single package, a series of hydrolytic enzymes whose operation, during the normal life of the cell, is in some manner held in check. If the assorted peptidases and nucleases of these lysosomes were released and permitted to act upon the proteins and nucleic acids of other subcellular structures, as indeed they do in post mortem autolysis, the cell must certainly die. But the manner in which these are held in check during the life time of the cell is completely obscure and it may be well to consider that occasionally the death of a

cell is a consequence of the release or activity of the enzymic components of these lysosomes.

It will be apparent that biochemistry has little to contribute, at this time, to definitive resolution of the problem at hand—the possible relationship between the influence of the delayed effects of radiation on life expectancy and the normal aging process. Several possibilities have been raised but definitive evidence is seriously lacking. On balance, it is the impression of this panel that the apparent relationship is completely fortuitous. The influence of radiation appeared most likely to operate by lodging in somatic cells defects in the genetic structure which provide the information for protein biosynthesis, defects, the expression of which results in cell death. Aging appears also to be a consequence of cell death with failure of replacement, but it appears to the biochemist, that the mechanisms underlying the time-dependent loss of the cellular mass of a multicellular animal are of a somewhat different nature than those which are operative in the post irradiated animal.

Physiologic aging and radiologic life-shortening

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THE PHYSIOLOGY COMMITTEE, consisting of Drs. Robert Akers, John Bakke, Cecil Entenman, Willet Asling, Hardin Jones, James Mead, Alvin Taurog, Donald J. Kimmeldorf, and Jonas Sirota, conducted two one-day conferences. It seemed to many of those present that adequate delineation and definition of the field of "physiologic aging" was required because of a common tendency to include within "aging" both longevity per se and pathological processes, such as atherosclerosis. The group felt that atherosclerosis, for example, may or may not be associated with aging, but is not an intrinsic part of physiologic aging. Most of the participants felt that physiologic aging should include only the inevitable, irreversible aspects of aging, unrelated to any known disease. If such phenomena as atherosclerosis, tumorogenesis, leukemia, and the like are included as aspects of aging, needless confusion must result. Thus, if one decapitates a mouse, its longevity is significantly decreased, whether decapitation be performed at 7 or 70 days of age. It seems unlikely, however, that sudden decapitation is related to physiologic aging. In contrast, the stress of a life in which the threat of sudden death is ever present must certainly influence the aging process.

If one accepts as a rough definition of aging a decrease in the total number of cells and in the functional capacity, as compared to the maximal number of cells and optimal functional capacity at some earlier period in the life of the individual, one may quantitatively approach the aging of any organ or tissue.

In attempts to establish possible parallels between physiologic aging and radiation injury, it becomes apparent that quantitative information concerning the total cellular components of specific organs and tissues is seriously lacking.

Ideally, one could prepare a chart in which a linear relationship could be established between radiation sensitivity, plotted on one axis, and susceptibility to physiologic aging, plotted on the other. As will be noted further, our present inadequate knowledge suggests something remotely similar to such a linear relationship in some tissues, but, in others, there appears to be complete lack of correlation. It seems conceivable, although it has by no means been established, that radiation might be used as a research tool in the over-all evaluation of the aging of some tissues.

HEMATOPOIESIS IN RELATION TO AGING AND RADIATION EFFECTS

A survey of the literature in this field indicates that there is no obvious correlation between hematopoeisis and normal aging (1–18). On the other hand, the hematopoietic tissues are exquisitely sensitive to radiation effects, exhibiting both inhibition of production of all cellular elements (especially the lymphocytes) and malignant changes (19). In this tissue, then, there would appear to be no obvious parallelism between physiologic aging and the effects of radiation. The review of Shields Warren is cited as a general reference in this field.

CARDIOVASCULAR SYSTEM

Major changes occur in the cardiovascular system as aging progresses (20). At this time, it is impossible to separate those changes that are attributable to pathological abnormalities, such as atherosclerosis, from those that are referable to physiologic aging per se. Heart muscle is quite radioresistant (21). Blood vessels are in the intermediate class with regard to radiosensitivity. Of the muscle coats, the endothelium is the most radiosensitive. Pathological changes induced in cardiac muscle by moderate amounts of radiation, therefore, are probably secondary to vascular damage.

CRYSTALLINE LENS

The crystalline lens is a tissue which generally undergoes rather specific functional change, with advancing years, leading to presbyopia. This is presumably related to changes in the interstitial tissue surrounding the capillaries that supply the lens (23). The capsule of the lens also becomes thicker and denser (24). The ciliary muscle also shows an increase in interstitial fibrous tissue, and the muscle fibers are thinner and denser than in the young. There is, however, no marked decrease in muscle power (25). There appears to be some correlation between the power of accommodation and longevity (26).

The lens is in an interesting area with regard to radiosensitivity. Recent work (27) has substantiated earlier observations (28) that the lens of the young animal is much more radiosensitive than that of the older animal and that irradiation through portions of the lens con-

taining significant amounts of nuclear material is required to produce cataract. In no organ is the relationship between the presence of cell nuclei and radiation effects more apparent.

Since cataract is a common manifestation of senility and since cataract can be produced in the young animal by radiation, the lens may offer an experimental approach to one specific correlation of aging with radiation effects. However, the details of neither senility-induced nor radiation-induced cataractogenesis are understood at this time.

THE LIVER

In the human, the best available data indicate that the weight of the liver increases steadily to the age of 20, remains at a plateau from age 20 to age 50, and then gradually declines (29). There is no definite increase in hepatic connective tissue in relation to aging (30). (Anyone who has attempted to masticate adult beef liver might dispute this.) A decrease in the efficiency of liver function has been reported with advancing years, but the studies have been insufficiently controlled (31).

It would appear, then, that the liver is an organ which does not participate significantly in the aging process.

Until recently, it has been accepted that the liver is a relatively radioresistant organ. Recent work of White et al. (32, 33) has demonstrated that moderate amounts of radiation, in conjunction with moderate protein depletion, produce liver damage in rats. Increased dietary protein protected against the radiation effect.

THE KIDNEY

Shock (34) summarized the problem of renal aging as follows:

> With increasing age there is a gradual diminution in renal function as indicated by reduced rate of glomerular filtration, reduced effective plasma flow, and a reduction in both excretory and resorptive Tm's. All the available evidence, up to the present time, indicates that the primary factor involved in the reduction in renal function is based on vascular changes. However, not all of the changes observed can be accounted for simply on the basis of a reduction in the number of vascular pathways and a structural narrowing of the vessels which remain. The vascular bed remaining in the aged kidney is capable of responding to vasodilators as effectively as the vessels in young kidneys. In addition to the anatomical narrowing and loss of vessels, there is present in the aged kidney a persistent vasoconstriction which tends to reduce further the flow of blood through this organ.

As in the case of cardiovascular changes, it is difficult to determine the extent to which these changes (some of which appear to be secondary to vascular alterations) are referable to physiologic aging and how much to pathological processes. Both morphologically and functionally, the kidney may be in excellent condition at an advanced age.

The human kidney is moderately radiosensitive, whereas the kidneys of several other species have been found to be more susceptible; both vascular and tubular lesions have been observed (35).

It seems possible that radiation may provide an experimental tool for evaluation of renal changes that bear some relation to those observed in the aging individual.

THE LUNGS

In general, vascular and parenchymal changes in pulmonary tissues tend to occur in association with the progression of the aging process. Concomitantly, there is a diminution in vital capacity, with the steepest decline occurring between the ages of 50 and 60 (36). Reduction in maximal breathing capacity also occurs (37).

Highly significant changes in the pulmonary vasculature may be induced by radiation.

At present, one can only speculate about the extent to which the fibrotic changes that occur with aging and after irradiation are secondary to the vascular changes or reflect primary change in pulmonary tissue. Consequently, one can only speculate also about possible parallelism between radiation effects and physiologic aging.

The lung is moderately radiosensitive (38). As in the changes associated with aging, both vascular and parenchymal changes are produced, and they will result in change in the functional capacity of the lung, if the radiation dosage is sufficiently high.

MUSCLE

Voluntary muscles. Striated muscle has been found to exhibit a decrease in cellular elements and a gradual decrease in functional capacity during the normal aging process (39). Similar changes can be produced by sufficient radiation dosage, although voluntary muscle is relatively radioresistant.

Observations in man, between the fifth and eighth decades, indicate that the work capacity decreases steadily with advancing years (40). Work output, as tested by standard ergometric measurements, at age 71 was 50% of that at age 41 (41). As nearly as it can be estimated, very large amounts of radiation would be required to produce such an effect.

Involuntary muscles. Essentially, the same statements as apply to striated muscle apply also to smooth muscle but even less adequate quantitative data, concerning both physiologic aging and radiation effects, are available.

CENTRAL NERVOUS SYSTEM

Decreased Nissl substance in the cytoplasm of Purkinje's cells in human subjects after the age of 50 has been reported (42).

Significant losses of cortical neurons are reported in aging human brains (43). A variety of changes in staining characteristics has been described. Some effort has been

made to differentiate true "aging" changes from those secondary to vascular lesions (44). This is difficult, and adequate quantitative evaluation of age-related changes in the central nervous system is not available.

Generally speaking, the nervous system is much less radiosensitive than its vasculature; hence, changes in structure and function will occur on this basis with amounts of radiation far below the levels required to produce histologic change (45).

THE SKELETON

Osteoporosis, the characteristic skeletal lesion associated with aging, is thought to result, in large measure, from changes in functional status of the endocrine organs. This does not lessen its possible inclusion as a true criterion of aging. Marked individual differences in the degree of osteoporosis at different age periods, however, suggest that pathological phenomena play a part in the development of osetoporosis, as in many of the other processes associated with aging. The bones of aged, growth-retarded rats were all demineralized, despite an adequate ration (46). Since the skeleton mirrors the net effects of the endocrines and of the nutritional status of the individual, it will be considered further later on.

THE SKIN

The skin is a mirror of age. Histologic studies indicate that the epidermis becomes a thin sheet (47). Interesting work has been reported recently in which it has been shown that changes in the elasticity of the skin appear to be correlated with fraying of the bundles of collagen, which has been observed by electron microscopy (48). This change is associated with changes in staining characteristics, and this may be a true criterion of aging; as such, it would lend itself to quantitative evaluation in association with functional tests of elasticity (49). A decline, with advancing years, in oxygen consumption by the skin has been shown (50).

From the standpoint of human studies, the skin presents the obvious advantage of availability.

The skin is relatively radiosensitive; recent studies (51) have indicated that epithelial damage is the primary effect. First there occurs a cessation of mitosis; then differentiation, with disappearance of the basal layer, is followed by further squamous metamorphosis, with acanthosis, dyskeratosis, and parakeratosis. Interesting histochemical studies are also being applied to the study of both irradiated and aging skin. It may be hoped that such techniques will establish the presence or absence of parallelisms between the changes associated with aging and with irradiation.

THE ENDOCRINE SYSTEM

As a system that affects all other organs and tissues and that, in many instances, can be evaluated quantitatively from the standpoint of structure and function, the endocrine system and its component members may lend themselves particularly well to evaluation of aging, on the one hand, and radiation effects, on the other. This subject will be dealt with briefly further in this report and in more detail elsewhere.

The Thyroid Gland

As seen in human post-mortem material, there is a reduction in size of all functional components of the thyroid in people from 60 to 80 years of age, and reduction in the number of mitotic cells. Also, there is an increase in the amount of connective tissue within the gland (52).

Rawson (53) cited studies of Rice (1938), showing that, from early adult life to age 80, the average weight of the thyroid gland decreased from 30 to 20 g, and that this was accompanied by characteristic changes in the histologic structure of the gland. This decline in the mass of secretory cells is in keeping with the well-known decrease with age in the basal metabolic rate, which is 3–5% lower for each successive decade.

The normal thyroid gland is relatively resistant to radiation effects (54, 55).

The Pancreatic Islets

By definition, diabetes is the end-point of progressive inadequacy of the beta-cell components of the islets of Langerhans. The incidence of diabetes or prediabetic states, therefore, should give some intimation of a tendency toward loss of functional activity in relation to age or radiation in these structures. In man, the peak severity of clinical diabetes is between 40 and 50 years of age, with a gradual decline after age 60 (56).

Correlation of structural status of the islet cells with the functional capacity is, to say the least, difficult and unsatisfactory. There seems to be no direct progressive relationship between decreasing function of the islet cells with increasing age, with particular reference to the period past age 60 in man. Presumably, the failure of survival of those hereditarily predisposed to diabetes may account for the apparent decrease in the incidence of diabetes after 60. Correction for this might establish a direct relationship between age and beta-cell inadequacy.

Islet-cell tissue is extremely radioresistant. However, hypoglycemia has been reported to follow irradiation of the pancreas.

It would appear, therefore, that no correlation is, at present, possible between radiation and aging effects on the pancreas. It may be that the superimposition of irradiation on hereditary predisposition may yield results of some interest.

The Parathyroid Glands

It is reported (57) that the parathyroids of the human male attain maximum size at age 20–30, after which a gradual decrease occurs, whereas in women, the maximal size is attained at about age 50. Histologic changes are difficult to evaluate at the present time. From the

functional standpoint, there is no suggestion that the parathyroids become less efficient as aging progresses. The usual indicators of parathyroid function, i.e., the concentrations of serum calcium and phosphate, are essentially identical in octogenarians and young adults.

The parathyroid glands are extremely radioresistant, as is evidenced particularly by the lack of any suggestion of hypoparathyroidism in individuals receiving large amounts of radioiodine for therapy of thyrotoxicosis. Thus, resistance to aging and resistance to radiation seem somewhat parallel in this organ, at least in a superficial way.

The Adrenal Cortex

Normal and even hyperplastic adrenal cortices have been described in persons past 90 (58). Further, the clinical use of adrenocorticotrophic hormones has well demonstrated that the very old can have excellent responses to administration of this trophic factor. However, Pincus, on the basis of urinary steroid excretions (59), and Samuels (60), on the basis of plasma levels of adrenal steroids, concluded that significant changes occur in the functional activity of the gonads and the adrenal cortex with advancing age. They also noted changes in the metabolism of steroids, as part of the aging process. Other workers have shown that urinary steroid excretion decreases progressively with age (61–63). It would appear, therefore, that the decreased functional capacity observed in the adrenal cortex with advancing years would probably be related to changes in the hypothalamic-pituitary control of the organ. None of these observations eliminates the possibility that qualitative changes in the nature of adrenocortical function may occur with aging, but this requires further investigation.

The adrenal cortex seems to be relatively resistant to the effects of radiation (64). Intensive radiotherapy to tumors in the region of the adrenal glands rarely produces demonstrable adrenocortical insufficiency. No adequate quantitative data are available regarding blood steroids and urinary steroid patterns under these conditions. These would be difficult to evaluate in any case because of the nonspecific "alarm reaction" referable to radiation effect on other tissues. Meaningful data must await long-term, chronic exposure to irradiation in conjunction with functional and histochemical studies in isolated glands.

Ovaries and Related Structures

The life pattern of the ovary is specifically associated with the aging process. Prior to puberty, it is functionally and histologically inactive. With the beginning of gonadotropin production, major histologic and functional changes occur in the ovary and in the tissues that are affected by ovarian secretions. After the menopause, despite continued production of gonadotropic hormones, the ovary becomes atrophic, and progressive atrophy follows in the tissues that are dependent on ovarian secretion. The administration of ovarian hormones corrects the atrophy in these dependent tissues. This sequence pattern has been elegantly demonstrated in experimental monkeys (65–67).

The rat's ovary at puberty contains 5,000 ova. Only a small proportion of these ova and follicles ever become mature. The number of eggs in the ovary of the aging rat progressively declines. Presumably, this is referable to decreased replacement, rather than to increased destruction (68). This reduction in functional capacity can be shown well by the decreased cornification of the vaginal mucous membrane as the climacteric progresses, even before the discontinuance of menstruation. Because of the marked "aging" of the specific tissues that are dependent on ovarian secretion, the question arises whether or not the aging of tissues that are not sex-linked is related to the lack of ovarian activity. There is no evidence to indicate that substitution therapy with one or more ovarian hormones can change significantly the life span of experimental animals. There is, however, some evidence to indicate that the local application of an estrogen results in younger histologic appearance of the aged skin (69, 70). There is abundant literature to indicate that osteoporosis is favorably influenced, although not eliminated, by estrogen therapy. Conceivably, other tissues, which are not considered sex-linked, may also be favorably influenced by such hormonal administration. Premature aging, at least with respect to the skin and bones, tends to occur in untreated patients with the syndrome of ovarian aplasia (gonadal dysgenesis). This can probably be prevented, or at least favorably modified, by adequate hormonal therapy.

The ovary is extremely radiosensitive, particularly in the newborn (71). Only the primitive ova and those of the secondary follicles are radiosensitive (72).

It would seem, therefore, that if one accepts the premise that ovarian hormones do exert a significant effect upon the aging, process, particularly in tissues such as skin and bone, that early irradiation could profoundly influence the pattern of aging in the individual.

Male Gonad and Related Tissues

The testes contain two major functional components: the interstitial cells, which produce testosterone or a related compound, and the seminiferous tubules, which produce spermatozoa. Both are dependent on pituitary stimulation for normal functional activity. Such stimulation begins at puberty and continues, to a degree, throughout the life of the individual.

In the male, the urinary 17-ketosteroid excretion, in a rough way, mirrors the functional activity of the interstitial cells. Studies have shown that there is a progressive decrease in such steroid secretion during each decade of life, with no suggestion of an abrupt change that might be considered to be analogous to the climacteric in the female (62).

The tissue anabolic effects of testosterone have been well demonstrated (73).

In view of the decrease in ketosteroid excretion, it might be thought that the decrease in the functional activity of the interstitial cells would result in a relative decrease in anabolism and increase in catabolism of tissues in general, particularly of muscle, on which testosterone exerts major effects. That this occurs is well documented, but, by the same token, it has also been demonstrated that the administration of testosterone does not overcome completely the progressive decrease in muscle functional capacity characteristic of aging. Presumably, this is referable to the progressive intrinsic decrease in number of muscle cells that is characteristic of the aging process.

As aging progresses, specific changes occur in the prostate in a large percentage of males. These changes are characterized by atrophy of muscle fibers, increase in connective tissues, and changes in secretory activity of the glandular epithelium. These changes are not necessarily associated with the reproductive potential of the individual. As far as can be demonstrated, testosterone administration does not prevent these changes, nor does it prevent the "benign prostatic hypertrophy" that is characteristic of a significant number of aging males.

Much additional information will be required before one can specifically relate changes in the gonadal activity of the male or female to the aging process in general. There would appear, however, to be no question that the anatomical and physiologic changes in both the male and female gonads are characteristic of the aging process.

The germinal epithelium of the male gonad is extremely radiosensitive, in common with other rapidly reproducing cells with a high ratio of nuclei to cytoplasm. The spermatogonia appear to be the most radiosensitive entities, whereas mature spermatozoa are quite radioresistant (74–76). As judged by histologic appearance, the interstitial cells also appear to be radioresistant. If radiation is to be used as an experimental tool, adequate studies must be performed to determine whether or not irradiated interstitial cells are functionally adequate, particularly in view of the relationship of testosterone to protein metabolism and age-linked processes such as osteoporosis.

In a recent study, Fleischmann (77), in attempting to evaluate the effects of radiation on testosterone-induced growth, observed partial inhibition of DNA formation in various tissues.

Pituitary Body

Anterior lobe. There is a slight decrease in gross pituitary weight in both men and women after age 40 (78). An increase of chromophobes in pituitary glands of old people has been reported (79), as well as an increase in the basophilic elements. Nevertheless, hypophysial gross weight is lower in old than in young adult animals (80). No definitive functional interpretation of the histologic changes is possible at the present time.

The anterior pituitary gland makes and secretes several hormonal complexes, including the growth hormone, which, to date, has not been separated from a "diabetogenic" factor; gonadotropins, which probably consist of three separate components, namely prolactin, thyroid-stimulating hormones, and adrenocorticotrophic hormones. All of these hormones appear to be secreted throughout life, with the exception of the gonadotropins, which do not put in their appearance until the prepubertal period, and which follow a reasonably predictable pattern of production thereafter.

In premenopausal women, there appears to be a progressive, fourfold rise in urinary gonadotropins between the ages of 10 and 50 (81). After the menopause, there is a still further increase in urinary gonadotropin, with peak value at 15 through 19 years after the cessation of menses. This peak is not maintained, but falls progressively throughout the next 20 years. In men, a slight increase in urinary gonadotropin occurs as a function of age. This increase is less than half that in women at the comparable age span of 10 through 49 years, and about one-fifth that in women over the comparable age span of 50 through 89 years.

Growth hormone can be found in pituitaries of old people, but the evidence indicates strongly that the production of this hormone is markedly diminished after puberty (82). More is known about the gonadotropins and the adrenocorticotropins than about any of the other hormonal factors because of more satisfactory assay procedures. No decrease in thyrotropin has been seen to occur in older individuals (83), but the evidence is not conclusive. The evidence for the possible decrease in corticotropin production with aging has already been considered.

One interesting observation which may be pertinent to the relationship of pituitary function to aging is referable to the clinical course of patients with congenital panhypopituitarism. In the characteristic clinical pattern which we have observed, until the age of 35 or thereabouts, the patient appears unusually young. Thereafter, one notes progressively accelerated "aging," e.g., wrinkling and drying of the skin, osteoporosis, and decreased general physical efficiency. This may occur to some degree even in those individuals who receive replacement therapy with thyroid and gonadal hormones, and cortisone, if the cortisone level is also deficient. Since the hormone which is not supplied in these patients, and which is very obviously lacking, is the pituitary growth hormone, one might wonder whether the lack of production of this factor might predispose specifically to premature aging. The current availability of potent human growth hormone may help to answer this question.

A corollary to this question is the possibility of favorable modification of radiation effects by growth hormone. One reported study (84) seems to answer this in the negative, at least in terms of survival under conditons of whole body irradiation.

The data available indicate that the anterior pituitary is quite radioresistant. Transitory disturbances in pituitary function may be associated with intensive radiation

therapy to a neoplasm of the skull. Destructive large doses of radiation are required to inhibit totally pituitary function for the purpose of favorably modifying neoplastic growth, diabetes, and other lesions.

Posterior lobe. Insufficiency of the posterior lobe of the pituitary gland is characterized clinically by diabetes insipidus. Some degree of dehydration is alleged to be a characteristic of aging. This dehydration of aging, however, is not characterized by the classical clinical features of diabetes insipidus. There would seem to be no reason, then, to believe that there is parallelism or a causal relationship between the progression of the aging process and the decrease in posterior pituitary function.

From data on human beings and experimental animals, the posterior lobe of the pituitary body would appear to be quite radioresistant.

Lymphoid Tissue

Because of the extreme sensitivity of lymphoid tissue to radiation effects and its high degree of metabolic activity, brief consideration will be given to its relationship to the aging process. The tissue is found throughout the body, but is present in major concentration in the lymph nodes, spleen, thymus, and tonsils. Lymphocytes are produced in great quantities in the lymphoid tissues and poured into the blood stream, a large proportion of them by way of the thoracic duct.

The physiologic functions of the lymphocytes require clarification. There is good evidence in support of the concept that lymphoid tissue is a source of antibodies (85–87). There is also evidence to indicate that small lymphocytes may differentiate into a variety of other types of cells (88–93).

The relationship of the lymphoid tissue to the aging process requires clarification, except for certain specific aggregates. The palatine tonsils attain maximal development about the 12th year, remain relatively constant until about age 40, then plateau to about 70 years, and thereafter decline further (94).

The thymus reaches maximal size before the 10th year, and then declines slowly until approximately the 20th year (95). Contrary to frequent statements, the thymus does not undergo complete involution in adult life.

The lymph nodes of old rabbits weighed only about half as much as at 5 months, when they attained maximal development (96). Significant changes occur in the histologic appearance of lymph nodes with advancing age in rats (97).

The spleen is the largest lymphatic organ of the body. The average weight of the spleen in the human being increases up to about 30 and decreases after 50 years of age (98). The lymphoid tissue component of the spleen diminishes progressively with age. However, the spleen of the old rat is larger than that of the young rat (99). Major histologic changes are seen in animals of different age groups.

In summary, it would appear that, at least in many locations, the quantity of lymphoid tissue diminishes as aging progresses. The significance of this to aging is unclear at the present time.

Lymphoid tissue is extremely radiosensitive, as is expected of cells that are rapidly proliferating and that have a high ratio of nuclei to cytoplasm. It is conceivable that the lymphoid tissue may lend itself to evaluation of possible parallelisms between aging and radiation effects.

DISCUSSION AND SUMMARY

As intimated in the introductory paragraphs, the present undertaking essentially represents a consideration of a few basic questions: *1*) What are the objective criteria by which one may measure physiologic aging? *2*) What is the mechanism of production of physiologic aging? *3*) By what objective criteria may one measure radiation effects on various tissues and organs? *4*) What are the mechanisms involved in the production of these effects? *5*) Are there sufficient similarities in any organs or tissues between the changes occurring with physiologic aging and the changes associated with radiation effects to lead one to believe that radiation could be used as an experimental tool for the study of physiologic aging?

No absolute answers have been provided for any of these questions. It seems necessary, at the present time, to indulge in extreme oversimplification and to regard a gradual decrease in total cell population in any given organ or tissue as a quantitative manifestation of aging. There is little question that qualitative changes in cells are also a part of aging and that, at present, we lack adequate means by which to measure such changes. By way of physiologic definition, one might regard aging as that situation in which in any given cell, tissue, or organ, catabolism prevails over anabolism, with resultant decrease in functional capacity of the part.

There would appear to be no question that radiation, in common with many other physical agents, if used in sufficient amount, can produce in any cell, tissue, or organ a state in which catabolism goes on at a greater rate than anabolism. Depending upon the nature of the cell, the cellular environment, and the quality and quantity of radiation, a variety of effects may be produced. These may range from slight change in functional capacity of the cell to inhibition of mitotic activity; to inhibition of all measurable chemical activity; to significant modification of structural composition, as measured by appropriate staining techniques; and to complete dissolution of the cell. Some cells, such as the lymphocytes, are extremely sensitive to the effects of radiation. Others, such as the cells of the adrenal medulla, appear to be extremely resistant. In some instances, there appears to be at least superficial parallelism between resistance to radiation and resistance to aging, or, on the other hand, sensitivity

to radiation and a predisposition to manifestations of early aging. In tissues in which such apparent parallelism exists, the possibility suggests itself that radiation might represent an experimental tool for the evaluation of the aging process. Not until the true biochemical significance of physiologic aging and of radiation effects is understood will one be able to state that such a working hypothesis is true or false.

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Meaning of experimental methods of changing rate of aging, with special reference to late effects of radiation exposures

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IN A SUPERB DISPLAY of scientific logic, Dickens described the process used by a newspaper editor to "bone up" on Chinese metaphysics: "he looked up, under C, China, and, under M, metaphysics, and then combined his information." In approaching the problem of "radiologic aging," I am afraid one cannot escape following a similar procedure, looking first at radiation, then at aging (or vice versa), and combining one's information.

To begin with, let us consider two definitions: Aging—the changes in an organism with the passage of time; senescence—the involutionary (or other detrimental) changes, in time, usually associated with the last portion of life. Only the first of these has definite objective meaning and is free from the somewhat subjective judgments of good or bad that beset the second definition. Hence, we shall confine our attention to aging, remembering that the end point of the processes of aging, at least in higher animals, is death.

Thus conceived, aging is an expression of the biological history of the individual. As such, no matter by what means it is measured, it is a function of both genetic and environmental factors. Almost by definition, then, we should expect to be able, within certain limits, to modify the rate of aging of an organism.

This assumption is borne out by experimentation. Let me cite a few instances of such effects: very young rats, when placed on a diet that almost completely arrests further growth by caloric restriction, have a life expectancy almost twice that of siblings reared on the same diet, but not restricted as to the amount of food intake.

Again, wild rats reared in captivity have a life span that is, at most, 60% that of rats of the same breed, but selected through a few generations for "tolerance to captivity." Conversely, the life span of domesticated rats can be markedly reduced—in the complete absence of traps, poison, or predators—merely by placing these animals in an environment more complex than a single cage.

In the bat, the nocturnal temperature controls life span within a factor of two by allowing or preventing nocturnal or seasonal hibernation.

Extreme control over life expectancy and many other so-called indices of aging can be exercized in colonies carrying such diseases as the endemic pneumonitis of the rat or the mammary carcinoma of the C_3H mouse by suitable choice of conditions of delivery and nursing.

In man, to give a final example, the courses of aging and the patterns of senescence are profoundly different among Western Europeans and among South African natives of the kwashiorkor belt—the difference being largely of nutritional origin.

Thus, the course of aging is surely subject to considerable modification, in the direction both of lengthened and of shortened life expectancy. This, however, is not all. The signposts of aging, whether they be diseases or changes in growth and function, provide a characteristic sequence of events by which we gauge the progress of aging in our subjects. Yet, the sequence—indeed the very occurrence—of some of these indicators is subject to environmental influences.

Caging, whether in single or multiple, simple or complex cages, can have the most far-reaching effects: in one strain of mice, mammary carcinoma is common, and a major factor in limiting life duration in singly-caged animals, but is reduced by the simple expedient of allowing two or more female mice to occupy one cage. In another instance, a series of convulsive seizures terminated life in a considerable fraction of old, singly caged mice, but was eliminated completely either by multiple caging, or by supplementing the diet with extra vitamin B_{12}.

Sex differences in life expectancy are prominent among all mammalian species that have been carefully studied, yet, in hypocalorically reared rats, these differences disappear.

Severe muscular dystrophy is among the major life-limiting conditions in a long-lived strain of rats. This "disease of old age" is eliminated or greatly reduced by vitamin E supplementation. In another colony, death of the males invariably occurred long after the disappearance of viable sperm from the seminiferous tubules. A

small extra ration of vitamin E eliminated this "senile" change without, incidentally, altering the mean life span.

The series of examples could readily be extended to include chronic infections, renal disease, skeletal changes, and many more. Such data, furthermore, could readily be supplemented by providing evidence of differential rates of aging among the various organs of a single individual animal.

The basic point has already been made: not only the course, but the very character of the processes of aging —or at least of the outward manifestations—can be varied within rather wide limits by a suitable choice of environmental conditions.

Thus, in any real mammalian population the concept of "normal aging," as well as the concept of "one single sequence of events" leading to senescence (basis for, or corollary of, the previous one) are largely artifacts owing to restricted vision. If so, any approach to our common problem from the point of view of "abnormal aging" may likewise serve only to generate an artifact.

Certain mathematical models have been constructed around formulas which serve well in the actuarial description of the age at death of various animal populations. Among these, the formula of Gompertz

$$G(N) = \log \frac{1}{N} \left| \frac{dN}{dz} \right| = Kz$$

enjoys pride of place, not only on historical grounds, but also because it provides a readily grasped picture of aging in terms of a force of mortality measured by $G(N)$. No matter how analyzed, this formulation describes aging in terms of a loss of ability to resist forces tending to unbalance vital homeostatic equilibria. This concept may well contain a profound truth. At the same time, however, it is so general as to be rather impervious to experimental analysis. Perhaps the most careful single study bearing on this function and its meaning shows that Gompertz's formula is a composite, reflecting the stepwise increase at widely different rates, of five or six major chronic diseases; in the particular rat colony under study, these include muscular dystrophy, periarteritis, myocardial degeneration, nephrosclerosis, and pituitary adenomas.

This is in line with our previous argument: once again, a unified concept of aging proves elusive, and, at present at least, not very helpful in planning experiments. The last-mentioned analysis, however, focuses attention on what may be the crux of our problem. If aging is viewed not as a unitary and inborn process, but rather as the accumulation, over the individual life span, of irreversible changes, then the experimental study of aging must deal with those separate processes by which an organism loses its ability to repair or to restore itself, or to rebalance its metabolism. Experimental modification of "aging" then becomes valid, and a useful tool, not by reference to some hypothetical "normal" course of aging, but only in proportion to its value in unraveling the mechanisms of injury fixation or of injury amplification which constitute the biological basis for the processes of aging.

It is in this light that it may be profitable to view the effects of ionizing radiation on the life span, and the course of aging, in mammalian species.

Consider first the dose-response relation: with the life span as an index, a near linear relation has been found between the dose of X-irradiation and life-shortening, provided doses causing acute reaction (death within 30–50 days or less) are excluded. The slope of this curve—and this will prove important—is by no means fixed but varies widely with fractionation. A somewhat similar linear plot is obtained if the incidence of leukemia is chosen as the index in a suitably susceptible population. And, to add a third example, the reduction of X-ray LD_{50} which, in rats, appears to persist indefinitely after radiation exposure to sublethal dose patterns, is, within the limits of our experiments, a linear function of total dose. All of these data, then, concur in indicating that the late effects of irradiation really exist, and that they are cumulative—although not necessarily always in a simple linear fashion. The published data do not suffice to allow extrapolation to very low doses, within the range of those now termed tolerance or maximal permissible doses, and we are free to conjecture what we please in this important region. (It is pertinent, in view of our previous discussion, to recall that there are good data suggesting an increased life span in certain mice and in Drosophila subjected to rather low dose levels of irradiation!)

A second manner of looking at the delayed-effects syndrome consists in cataloguing the incidence of a number of visible lesions. These include certain malignant lesions induced directly or indirectly by the radiation exposure, or lesions in some way made to appear at an earlier age in irradiated animals than in unexposed controls—graying of hair, cataracts, and—possibly—nephrosclerosis in mice. On the whole, the obviousness of these lesions has led us to give undue weight to them in describing the post-irradiation animal. A more adequate description should also include reduced body weights, a wide variety of vascular lesions, atrophic changes in certain organs, and a life span that may be considerably reduced with no higher tumor incidence than in controls of the same age. The clinical picture that emerges does not reflect direct effects of ionizing radiation on a specific organ but, rather, seems to be an expression of the generalized metabolic disturbance resulting from the disruption of numerous specific homeostatic systems. An excellent illustration of this point is the lymphomatosis of the $C^{57}B$ mouse; it can be traced back to the thymus but its development does not require irradiation of this organ itself—a normal thymus, transplanted to a thymectomized irradiated mouse will give rise to the neoplastic condition. Several other examples of such indirect effects resulting from disrupted homeostatic systems have been observed; many more probably remain undetected.

A third way of viewing the late effects of radiation exposure is in terms of the resultant loss of physiologic reserves—a loss that, in the ordinary course of events, goes

undetected until it is unmasked by a specific challenge. An example of this, alluded to previously, is the permanent decrease in radiation resistance of the previously X-irradiated rat. This effect not only provides a measure of the irreversible injury, but also provides an index of the loss of some of the reserve capacity of the bone marrow—a loss that remains occult until uncovered by the second irradiation challenge. A somewhat similar phenomenon is the biochemical lesion resulting in impaired nitrogen utilization, barely suggested by the weight deficit of the irradiated animal, but unmasked and sharply brought out by various types of undernutrition-realimentation cycles.

Yet another point of view is that radiation exposure might elicit premature aging. Like the concept that senescence is owing to testicular failure, or to thyroid dysfunction, this point of view seems to me to be a half-truth. For example, the radiation resistance of mice, as measured by LD_{50}, decreases with age. If, now, a young mouse has been irradiated in a manner that allows its survival through the acute reaction, two things have happened: its life expectancy has been reduced, and its radiation resistance has decreased. The amount of life-span reduction, however, is far smaller than would be predicted from the change of radioresistance in view of the normal age-radioresistance curve. Indeed, the values differ by a 100%, so that clearly the "radiation-aged" mouse is not equivalent to the "shelf-aged" mouse.

Calling attention to the very incomplete parallelism between natural aging and the late effects of radiation exposure, one should not, however, obscure the fact that, in a broader sense, the two processes have much in common: both involve the accumulation of irreversible lesions by the organism. There is good reason for feeling that the mechanisms of injury-fixation in the irradiated animal are not unique.

Thus, the consideration of the late-effects syndrome from this point of view, constitutes a further and, in many ways, the most attractive aspect of our problem: the exploration of the mechanisms by which injury owing to ionizing radiation can be fixed in mammalian tissues, and by which such fixed injury can be amplified in the course of time to give rise to overt delayed effects.

There are several lines of experimentation which spring from such a point of view. Thus, one may consider the late-effects syndrome to be an extension, in time, of the acute radiation effects and search—in the events of the first few weeks after radiation exposure—for injuries that will never be fully repaired. In some cases this approach seems to hold real promise. Thus, the bone-marrow defects, certain endocrine effects, and those aspects of the delayed-effects syndrome that are maximized by very limited fractionization of the total dose would seem to fall into this category.

From another angle, this problem of injury-fixation may be approached in terms of possible histopathological mechanisms that can result in permanent tissue alterations. Examples that come to mind here include the deletion of stem-line cells in proliferating tissues; the incorporation into structural elements of faulty products of cellular metabolism; the alteration of the biochemical, hormonal, or physical balance in key organ-systems under conditions in which return to the former equilibrim either becomes impossible or is achieved by a series of poorly damped oscillations.

At a cellular level, the most obvious point from which to take off is the demonstrated fact that radiation can induce mutations as well as chromosomal aberrations, and that in tissues like the liver, in which the normal mitotic rate is rather low, one can demonstrate a very high proportion of abnormal mitoses—and, by inference, an even higher proportion of cells carrying induced mutations. This is accomplished by eliciting a wave of mitoses by partial hepatectomy, months after the last irradiation. The remarkable fact here, perhaps, is not so much that some anomalies are observed, but rather that such a tissue can function and can regenerate a functional tissue mass that is not readily distinguishable from normal, not irradiated tissue. Since the basic phenomenon almost certainly is not confined to the liver but occurs universally in all tissues, considerations of cell population dynamics—i.e., of changes not so much in individual cells as in the kinds and numbers of cells that make up a given organ over the course of its lifetime—would seem a most fertile avenue of approach to the study of late radiation effects. Surely, if there is any parallelism between radiologic and physiologic aging, the fluctuations and the drift in cell-aggregates in the various organs would be a sensitive index of the degree of convergence or of divergence of the two process sequences.

In all that has been said so far, the tacit assumption has been that the radiation exposure is of such magnitude that subsequent changes in the clinical history of the exposed subject are (hopefully!) clearly traceable to the radiation exposure as their cause. When, however, one directs one's attention to progressively lower levels of radiation exposure—a matter of prime concern to many concerned with defining tolerable exposure levels for all sorts of different circumstances—these relatively dramatic sequences fade until they are no longer readily distinguishable from those that are "normal" for the changes in the physiologic aspects of an individual organism with the lapse of time. Yet, it may be only at these levels that radiologic "aging" and physiologic aging truly approach each other.

Clearly, for such effects, the proper object of study ceases to be the individual (where the effects have become invisible) and becomes the population. It is at this point, also, that our previous arguments regarding the nature of the aging process are properly brought to bear upon the problem of delayed radiologic effects. We made the point that aging is not a unitary process, but a compound sequence of events, subject to qualitative as well as to quantitative modification by environmental factors. At the radiation levels we are now contemplating, all that needs to be added is that radiation is—or most likely will turn out to be—one of the environmental factors that can modify aging. Such a conclusion, far from being

sterile, points the way to the experimental approaches that can provide the data for sound evaluation of the effects of radiation on aging in exposed populations. We concluded previously that no one factor of a nonlethal environment can be said to change the life history of any animal in any specific way unless the pattern (or the sequence of patterns) of all other parameters has been fixed and well defined. As a corollary, it follows that, if one wishes to single out one such factor and make meaningful statements about its effects on aging—or about any other, similarly comprehensive function of the test population's biologic aspects—one must test its effects not in one or two, but in a carefully chosen array of arrangements of the remaining major environmental parameters. Only from such complex studies can we derive information that will be objectively correct, and will also provide a meaningful basis for the kind of value judgments which, in the last analysis, are the reason for the public's concern with the problem before us.

In making this final plea for what amounts to an ecological point of view in regard to the subject of radiation and aging, I am quite aware that I am pleading for future work, rather than pointing up present trends. These, I suspect, have by and large hewn close to the traditional line of laboratory research, dealing with mechanisms rather than with attempts to substantiate quality judgments.

Of equal importance with all this, however, it seems to me, is the fact that this problem area, perhaps more than any other in the past half century, serves to focus attention on the need for, and certain specific means towards, bridging the methodological gap between aspects of biology which have remained largely observational and aspects which are intimately associated with laboratory experimentation. Here, as elsewhere, radiation biology may well make a major contribution to the development of biological thought as a whole.

Estimation of radiation effects at small exposures

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ESTIMATION of physiologic effects of low-level irradiation necessitates a wide system of observation; the problem involves detection of long-range changes as distinct from the acute effects of exposure. Alterations in function should extend over the remainder of the life span, if no recovery or only slight recovery takes place in the system; there is some question, however, as to whether the alteration may be discernible in the steady-state levels or in the seldom measurable functional reserve of the system.

At the present time, knowledge of latent radiation change is virtually limited to the higher ranges of exposure. Thus our problem is a double one: not only to quantify long-range effects under these conditions, but also to determine whether they exist and are measurable at smaller exposures.

In the survivors of the explosions of the atomic bombs, there is evidence suggesting numerous small deviations in the average value of physiologic measurements. Although extensive in the characteristics affected, they are slight in magnitude, averaging only several percent deviation in mean value in association with several hundred roentgens of radiation exposure. The circumstances of observation are such that one cannot be certain whether this is a cause-and-effect relationship due to radiation, rather than an association based upon the method of collection of cases or upon the effects of other deteriorative forces also associated with the bombing.

For the purpose of planning additional studies, it seems proper to assume that the effects observed are the result of radiation exposure, so that we may proceed to test this hypothesis in other ways. It is well to point out that the evaluation of latent radiation effects is dependent upon the grouping of 500–2,000 cases for each statistical measure. If we are to consider exploration of the problem of whether these same physiologic changes occur to a reasonably proportional extent at one-tenth the above radiation exposure, then we will be looking for differences of the order of 0.5% between the irradiated and the control populations. Under the most favorable conditions, the sample size necessary to establish so small a difference at a reasonably high level of confidence would be about 100,000 persons. Thus, the sample size has risen from 1,000 to 100,000 in the dropping of exposure to one-tenth its former value. Indeed, this modification presents some extraordinary difficulties, and we must be very wary, as we proceed, to determine in advance of our observation whether the test-system used has a reasonable chance of resolving the issue concerned. It will be very little gain over present knowledge in establishing, for any low-dosage situation, that no measurable effects occurred when, indeed, we could deduce that from the outset. If effects occurred exactly as predicted, the measured difference likely is too small to have sufficient statistical significance to permit dismissing these small average effects as being of no consequence, when compared with the known normal range of function; for we are looking for clues to explain the linkage between the acute effects of radiation exposure and the late manifestations of degenerative disease. Even very small effects, acting over a life span, may be the etiologic agency of the long-term response to radiation. It is also possible that some of the manifested change may be expressed in wider dispersion of the range of distribution of values, rather than in alteration of the average value; to investigate these effects would require an analytical system sensitive to a number of such possibilities.

It is probable that certain effects of radiation exposure, which are of greatest magnitude, may be selected. One such criterion is the disturbance of blood cells. Not only is the marrow system changed, but there is also a prominent shift to high leukemia risk and possibly all of the blood dyscrasias are similarly elevated in risk. There is also evidence that the enzyme content of blood cells may be altered. Presumably, if we could measure the functional characteristics of individual cells, there would be present an increased incidence of somatic mutations. This kind of observational system may be much more to our purpose, because these changes are all-or-nothing; and, if they exist, there is just as much chance per cell of recording the fact, regardless of the dose of radiation exposure to the individual. It is possible that the insignificance of some of the changes in steady-state systems is due to the fact that only a small fraction of the

cell population is altered and that, in average function, at least, the unaltered cells, with normal functional resources, are providing for metabolic processes. Thus, it is useful to shift our attention, whenever possible, to the molecular or cellular level, because of the expected all-or-nothing effects, which are measurable and which can, in turn, be used to estimate the change in physiologic reserves as a consequence of radiation exposure. For such a system of observation, we could use the blood cells and the cells of the skin and mucous membranes. Although it would be interesting to see the effects on other cells, I think that these cells are adequate for testing the hypotheses involved; routine biopsy of other tissue is, moreover, traumatizing. Besides, we have scarcely begun a study of the human cells immediately available for our study. I believe that it is possible that the study of these cell populations will be profitable in showing a differentiation associated with mutation; this has important bearing on aging as well as on radiation effects. It is, indeed, in this area that one might expect the greatest simulation of aging by radiation exposure. The process of aging is already known to be associated with a gradual increase in islands of cells that appear to have different growth and pigmentation characteristics, as though they had been sown on an agar plate. The nails, too, show ridging and fraying in streaks, as though there has been a random change in the cellular composition from the uniform bed that characterizes young individuals.

In order to test the hypothesis that radiation effects simulate those of natural aging, we first need to consider the probable magnitude of the factor for conversion of exposure into aging equivalents. It is estimated that 1 r is equivalent to a loss of 5–10 days of the human life-span scale of time. Natural radiation exposure, then, which is an accumulation of about 5–10 r over the life span, may have accounted for a reduction in life span of some 25 to 100 days, if the exposure to radiation at such dose rates produces effects proportional to exposure effects from several hundred roentgens administered in a short time. If we consider specific physiologic changes that are known to be related to aging, we may then ask how much chance we have of observing a drift in that function as a result of an age change of 25 days, 1 year, or 10 years. This corresponds with the aging effect of radiation exposures of about 5 r, 50 r, and 500 r, respectively. Most of the changes in average physiologic function observed after irradiation at low or moderate dosage levels have been slight, but so is the average physiologic change in the usual systems associated with aging, when followed over a small fraction of the life span. It is necessary to observe the entire span of adult life to see the changes that occur with age in body composition, in cardiac output, or in renal function. An extraordinary number of cases may be necessary in order to establish with reasonable statistical confidence that a physiologic system differs from the normal by 10 years of physiologic aging. As the stimulus diminishes toward the range of natural exposures, the reduction in the effects expected increases the number of cases required to test the hypothesis by a power function of the relative reduction in the exposure. The following table shows significant changes in average life span, or some measure related to it.

Numbers of animals in relationship to exposure in order to test that radiation reduces life expectancy at the 1% probability level (control population very large)

Dose in r	Expected fraction of life span lost	No. of animals required
1000	0.3	6
500	0.15	10
100	0.03	900
50	0.015	3,000
5	0.0015	185,000
1	0.0003	4,600,000

From this table, which is equally applicable to mammals in general (on a relative time scale), it is possible to estimate that conventional approaches to experimental observations, in terms of numbers of animals, are reasonable down to about 100 r. Below this dose, the effort becomes difficult or, finally, impossible. Although this calculation is derived from the statistics of the sum of individual deaths, it is also likely to apply, in a general way, to a chance of determining differences between the means of physiologic steady-state systems that are altered by radiation exposure. These have been discussed earlier.

The one system that appears to be inherently sensitive to radiation comprises the blood-forming tissues. After irradiation, the numbers of blood disturbances and leukemias appear to be greater than would have been estimated from consideration of the increase in these diseases with age and the general relationship between radiation exposure and degenerative disease. Some have argued that this observation tends to differentiate radiation effects from aging. However, the acute radiosensitivity of these cells is also greater than that of the average body cell; therefore, it is reasonable to suggest that these tissues may be inherently more sensitive to radiation at any level than others in the body. On this basis, the only revision of viewpoint required, so far as specific effects on the blood-forming system are concerned, is modification of the numerical value of the conversion factor between radiation dosage and aging.

I would expect that further study of the contrast between radiation effect and aging may not lead to a true segregation of the two. Both, as we now observe them, are end-effects. It is possible, and even probable, that to some extent these end-effects of aging or of radiation exposure plus aging have common pathways and common mechanism, and that to some extent they have been produced by different events leading eventually to the same end. It is also possible that we are too crudely establishing end-states as diagnostic entities; to some extent, pathologic classifications are fixed, and each tally must enter one of the classical "pigeon holes." If we had a more refined system of classification, we

would have a greater chance of differentiating between radiation exposure and natural aging. The gross similarities between radiation effect and aging are now so evident that it seems unlikely that this general notion would be abandoned. Study of these problems offers a special opportunity to examine both the process of radiation damage and aging, with recognition that minor differences between the two are just as important in establishing understanding of both as are the points of strong similarity.

It is likely that the attention devoted to the blood-forming system will be relatively more fruitful than the study of general physiologic function, because tentative evidence indicates that radiation-induced changes in this system are greater by a factor of 5–10 than in other parts of the body. It is even possible that average changes observed in the body as a whole associated with radiation may be secondary manifestations of changes in the blood-forming system.

Of the possible systems that may be inspected at low exposure, a few may not entail the difficulties inherent in statistically validating a concept, when the effects are small. Among these, as I have noted might be the all-or-nothing changes, at the cellular level, resulting from both radiation exposure and aging. A second possibility is reliance on such systems as growth. In this instance, it is possible to evaluate the status of each individual with precision, and, therefore, be able to test for the effects of radiation with much more chance of significance. I might note that growth effects were proved in relatively small population samples of the children exposed to thermonuclear explosions in Hiroshima, Nagasaki, and the Marshall Islands.

Relation of gonadal maturation to length of life in Pacific salmon

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THE PACIFIC SALMON lends itself in a unique manner to experimental studies on influencing the life span in a vertebrate form. All five species of the genus *Oncorhynchus*, found along the Pacific coast, have sharply limited life cycles, which range, typically, from 2 years to 4 or 5 years, depending on the species. These salmon are anadromous. They descend to the sea as juveniles and, after 1–3 years of marine life, migrate up the rivers of their origin, where they spawn and then deteriorate rapidly, and all die within a week or two. Why do these fish all succumb after their first reproduction? This is a phenomenon unknown among mammals and rare among fishes. Is it due to shedding of a large mass of sex products or to some profound physiological change associated with sexual maturation, or is the life cycle of each species of Pacific salmon biologically fixed, spawning constituting the final phase?

As an initial approach to the problem of the nature of the postspawning death, a study was made of the histological changes that occur in the various internal organs of the spawning salmon, beginning with the pituitary gland. Accompanying development of the gonads, the pituitary gland exhibited evidences of markedly increased activity, followed at full maturity and spawning by extensive degenerative changes which were, except for greater severity, much like those found in the pituitaries of senescent mammals including man (1, 2). Most of the other internal organs also showed deteriorative alterations, some of which resembled senile changes occurring in mammals. In contrast to this general degeneration and atrophy, the adrenocortical tissue was found to be in a state of marked hyperplasia, although, at spawning, degenerative changes had begun. The effects of hyperactivity of the adrenal on the organs and tissues of these salmonid fishes is being investigated.

To obtain further light on the relationship of gonadal maturation to termination of life in the salmon, two experiments were undertaken. One was to observe the effect of loss of sex products in fish with accelerated sexual maturity (3). In certain salmon populations, precocious sexual maturation occurs in a small percentage of male fish at 9–10 months of age. These juveniles develop an enormous mass of gonadal tissue—up to 25% of the body weight, which is a much larger ratio than in the adult salmon. A considerable number of these precocious fish, after artificial spawning, were kept under observation together with sexually immature controls. At the end of 5 months, the test fish had survived just as well as the controls and showed renewed spermatogenesis. Age appears to be the deciding factor in determining death or survival in this species of salmon following spawning.

The second experiment consisted in castrating a large number of juvenile salmon at 2 years of age to determine whether prevention of development of the sex glands would result in prolonging the life cycle (4). The species used, the kokanee (*O. nerka kenneryli*), lives in fresh water and has a 4-year life cycle. Within 2 years, i.e., at the end of the fourth year of the experimental fishes' life, most of the control (unoperated) fish showed evidence of sexual maturity and died. Likewise, a number of the castrated fish, mostly males, developed similar morphological changes and died. Autopsy of these castrated salmon revealed regeneration of the gonads. In many of them, the amount of regenerated testicular or ovarian tissue was very small, but was sufficient to initiate the series of changes leading to degeneration and death. Thus, the development of a large gonadal mass was not the important factor in the demise of these fish. By the end of the fifth year, all the control fish had died. A small group of the castrated fish remained; some of these subsequently regenerated gonads and died. At the time of writing, 9 castrates, 7 females, and 2 males are alive and healthy, well along in their seventh year; this is 1½ years after the death of the last control and 6 months after the last castrated fish had regenerated gonadal tissue. From these results, it seems fair to conclude that *1*) the life span of the remaining castrated salmon has been prolonged, and *2*) that the death of the Pacific salmon is due to lethal physiological disturbances initiated by maturation of their gonads.

ADDENDUM

Since this manuscript was sent in for publication some considerable time ago, the above described experiment on Kokanee Salmon has been terminated. The last of the castrated Kokanee died at

the age of 8 years and 6 months, having lived more than 3½ years longer than the last surviving uncastrated control. A complete description of the experiment will be published in the April 1961 issue of the *Proceedings of the National Academy of Sciences*. Further studies bearing on changes in the activity of the adrenal cortical tissue and modifications of other organs in the spawning Pacific Salmon have published in *Endocrinology* 65: 225, 1959 and 66: 222, 1960.

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Cellular aspects of irradiation and aging in mammals[1]

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THE AGING PROCESS is one of the great biological mysteries. Like chronic, low level irradiation in mammals, it produces degenerative effects which are obscure in their essential nature. These two processes share a number of apparently fundamental parallelisms: the mortality rate of a population which has aged or has suffered significant exposure to low level doses of ionizing radiation increases in a fashion which is a direct function of the amount of aging or irradiation experienced. In both cases it is so far impossible to find any particular organ, tissue or physiological function in which a primary defect has been lodged, but rather does there seem to be a generalized loss of physiological efficiency in many or most tissues. Finally, the action of both agents is accompanied by an increase in the frequency of malignancy.

More fundamental analysis of the nature of these two processes has been impeded by the lack of sufficiently penetrating tools for studies on mammalian cells. With the development in recent years of more quantitative methodologies (1) such as cell plating procedures which permit measurement of the number of reproductive cells in any population; simple methods of clone isolation; the ability to establish long term cultures of cells taken from any individual; the ability to follow the life cycle of any cell by the use of agents like thymidine labeled with H^3 or other isotopes (2, 3); visualization and analysis of the chromosomal constitution of mammalian cells; attainment and measurement of synchronization of cell populations (4, 5); and the beginnings of study of processes like specific enzyme induction (6), much more rapid progress may be expected.

There are three general types of response to the action of ionizing radiation which all living cells display: a reversible lag in the onset of reproduction, an irreversible killing, i.e., destruction of the ability to multiply indefinitely, and the introduction of random mutations which may involve only single genes or produce gross alterations in the structure of the chromosomes. Some progress has now been achieved in the study of all three of these effects in mammalian cells.

Study of reversible mitotic lag induced by X-irradiation of mammalian cells is particularly convenient with cells like the S3 HeLa which is an aneuploid, hyperploid cell that initially arose from a human cancer of the cervix. This cell has a survival curve (7) which exhibits an initial shoulder so that, by the use of doses of up to about 85 rads, almost no irreversible cell killing occurs to complicate the situation. Doses of 30–85 rads produce profound, though temporary, effects on the cells' life cycle. The mitotic index at first drops sharply; then recovers; overshoots, reaching values twice or three times normal; after which it again falls toward the normal value. Analysis of the kinetics of this action with H^3-labeled thymidine revealed that neither G1, the postmitotic period of the life cycle, nor S, the period in which DNA is synthesized, are affected by these doses. However, a strong temporary inhibition of G2, the premitotic interval, is produced by such irradiation at a point approximately 1–3 hr before the onset of mitosis. Cells pile up behind this block so that the mitotic index at first falls. After release of the block a much larger number of cells then enters mitosis, producing a burst of mitotic cells (8).

Preliminary studies with normal, diploid, human cells have shown that they, too, are extraordinarily sensitive to the production of reversible mitotic lag at very low doses of radiation and that, like the heteroploid S3 cell, a block in the G2 premitotic period is induced by irradiation. However, in contrast to the behavior of the hyperploid, malignant cell, the normal human cell also displays a second radiation block in the G1 postmitotic phase. This difference in radiation sensitivity of these two cells is being studied further.

Irreversible killing in human cells irradiated in vitro occurs with doses somewhat larger than that required with reversible mitotic lag. The mean lethal dose of the S3 cell is 135 rads (7). Determination of this figure was made possible by means of quantitative cell culture methodologies which revealed the error of previous ideas that the mammalian cell required many thousands of roentgens before it was irreversibly damaged (9–11). The fact that

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the mean lethal reproductive dose is in the neighborhood of 100 rads for all mammalian cells so far studied has greatly clarified the mechanism of a number of different aspects of the mammalian radiation syndrome.

It is important to determine whether this high susceptibility to reproductive death of irradiated mammalian cells is characteristic of all the cells of a population or only that fraction which is in mitosis at the time of irradiation. The greater sensitivity of cells in the mitotic state is a well-known phenomenon. Analysis of the mitotic distribution in cell populations demonstrated that only 3–4 % of these cells is in mitosis at any one time. Nevertheless, administration of single doses of irradiation within a period of minutes or seconds results in survival curves for the population as a whole which continue to fall uniformly down to a point where less than a hundredth of a per cent of the original population is able to form colonies. Hence, the conclusion can be drawn that the mean lethal doses of 135 rads for the S3 cell and 86 rads for the normal, diploid, human cell are essentially those of interphase cells. By analogy with studies on other organisms, one might expect that cells with condensed chromosomes in or near mitosis would be even more susceptible to reproductive death following irradiation. Such effects have recently been observed by Tolmach (personal communication).

It is of critical importance to know whether this high radiosensitivity of the mammalian somatic cells, which is several orders of magnitude greater than that of other single-celled organisms like bacteria and protozoa, is also exhibited in the body or whether it is an artificial circumstance developed by in vitro conditions of cultivation. Three different approaches have recently demonstrated that cell radiosensitivity in vivo and in vitro are the same within the present limits of experimental uncertainty. It has been demonstrated by Hewitt and Wilson (12) that when leukemic cells taken from an irradiated mouse are injected into a normal mouse, the number of cells required to transmit the disease to the new animal increases directly with the amount of irradiation given to the donor animal. In this way, they were able to titrate the survival curve of the leukemia cells irradiated in vivo. The results demonstrated a mean lethal dose very close to that which we have found for a variety of mammalian cells in vitro. The same kind of conclusion with respect to a different cell type is obtainable by analysis of the survival of fertilized mouse ova in females irradiated shortly after fertilization. We calculated that the experimental survival observed by Russell and Russell very closely paralleled that predicted on the basis of the in vitro survival curve (13). Finally, in a particularly elegant series of experiments Till and McCulloch (14) demonstrated that the radiosensitivity of mouse bone marrow cells is virtually identical in vivo and in vitro exhibiting a mean lethal dose of 115 ± 8 rads. These different lines of evidence show that at least a significant number of different rapidly dividing somatic cells in the body exhibit a high radiosensitivity of the same general magnitude as that shown by mammalian cells in vitro.

A variety of considerations which have been discussed elsewhere (1) has led to the conclusion that, of the mammalian cell structures, the chromosomes are plausible candidates for the major site of significant radiobiologic injury for doses less than 200 rads. Among the most convincing data in this connection has been a demonstration that mammalian cells whose mean lethal dose is 85 rads display significant numbers of visible chromosome aberrations when irradiated with doses equal to or less than a mean lethal dose (15). Thus, analysis of chromosomal breaks, deletions and abnormal recombinations in diploid human cells irradiated in vitro and scored for various periods up to several days after irradiation, led to a gross average figure of 50–60 rads as the dose producing one visible chromosomal aberration per cell. This figure is important, not as an absolute number, but rather as a general index of the high degree of chromosomal radiosensitivity in such cells. It must be remembered that present methodologies permit only the scoring of chromosomal aberrations which are visible at mitosis. All of the complex, and as yet obscure, events of the lag period intervene between irradiation and the subsequent arrival into mitosis of the cell population under study. It has long been recognized that chromosome breaks can rapidly be rejoined inside a cell so that visible evidence of damage only remains in those cells which have failed to reseal completely, or where breaks have recombined in a sufficiently anomalous fashion to yield cytologically recognizable aberrations. Estimates of the actual numbers of chromosome breaks that must have been introduced by such an irradiation process have been made and the number arrived at is approximately 10 or 20 times that observed by the scoring of subsequent mitoses (16). Moreover, a certain proportion of the cells which have been killed reproductively by irradiation may not even reach a single mitosis afterwards and so fail to make their contribution felt. Thus, we have shown that in experiments testing the yield of chromosome aberrations in diploid human cells, a steady decrease in efficiency was obtained for samples incubated at progressively greater periods after irradiation. The maximum yield which we have observed in normal human cells, irradiated with 82 rads, occurred within the first hour after irradiation. Analysis of 20 consecutive mitoses revealed 86 clear aberrations, corresponding to a value of 19 rads as the average chromosome breakage dose. The critical nature of these time relationships and the fact that mitotic lag is also involved in this process, makes it necessary to be cautious in attempting to calculate absolute values of chromosomal breakage efficiency. Studies are now in progress which, it is hoped, will make possible such detailed calculations and experimental unravelling of the course of chromosomal events that occur in the interval between cell irradiation and the subsequent achievement of mitosis.

On the basis of various lines of evidence, an explanation has been attempted which would explain both mitotic lag and cell reproductive deaths in terms of radiation induced chromosomal damage (17, 18). The chromosomes carry on three functions in the cell: *a*)

Each chromsome consists of a linear array of DNA molecules which constitute the genes which send off into the cytoplasm messenger RNA molecules containing the information leading to the synthesis of specific enzymes and other proteins needed by the cell. *b*) Each chromosome duplicates itself prior to cell division, and this replication of the genes is the central process of biological reproduction. *c*) Finally, each chromosome undergoes a complex process of coiling to form a highly condensed structure. This complex, reversible, mechanical transformation of the chromosomes is a concomitant of mitosis and meiosis and makes possible an equitable distribution of the chromosomal complement to each of the resulting daughter cells.

Demonstration that the reversible mitotic lag in the S_3 cell occurs because of a block coming just before mitosis suggests a metabolic basis for this inhibitory action. This is the period during which chromosome condensation occurs—i.e., in which chemically mediated mechanical forces bring about supercoiling of the chromosomes which decreases their total length by more than 99%. Introduction of chromosomal breaks or other defects might interfere with this process. Even very small radiation-induced structural defects, like a break in a single DNA strand, could conceivably become translated into a complete discontinuity of the chromosome by the action of the mechanical forces arising during this condensation period. The resealing of these breaks may be the necessary process responsible for the block in the G_2 period. Presumably only cells in which these breaks have been resealed in such a manner as to permit the orderly process of condensation to proceed can enter into mitosis.

By this picture, small doses of radiation, i.e., 75 rads or less, would introduce a relatively small number of primary chromosomal lesions in each cell. These could reconstitute normally, so that only a temporary mitotic lag would have been exhibited. While occasional gene mutations might result from such actions, the probability that these could affect reproductive survival is very small for a cell of ploidy equal to or greater than two, as is the case in mammalian cells. Larger doses of radiation would introduce so many chromosomal lesions as to provide a high probability for irreversible damage through processes including abnormal recombination of chromosome fragments leading to forms mechanically unable to continue condensation or possessing dicentric chromosomes which may prevent completion of mitosis; or through the production of gross deletions and rearrangements which could cause lethal genetic imbalance in the resulting cell. This picture which as yet can only be regarded as a working hypothesis, provides a unitary mechanism underlying all three of the basic actions of irradiation on cells—mutation, reversible lag and irreversible reproductive death.

The high radiosensitivity of mammalian chromosomes which has been here discussed is in marked contrast to the extraordinarily low sensitivity of physiologic cell structures and enzymatic functions which have been so carefully studied by many investigators (19). Many experiments on a variety of enzyme systems isolated from cells or studied in cell homogenates, whole cells, or even intact tissues, have failed to show any significant effect for doses below hundreds or thousands of roentgens. We have demonstrated that mammalian cells in vitro can continue to biosynthesize and carry out a wide range of metabolic functions for long periods, even after exposures to many thousands of roentgens (7). One mechanism in particular has been frequently suggested as a possible site of the primary radiation damage to mammalian cells, i.e., the regions of the cellular membrane responsible for the active transport processes. Indeed, in microorganisms the failure of these transport processes has been demonstrated after irradiation experiences comparable to, or even smaller than, the mean lethal reproductive dose. We have carried out such experiments in mammalian cells, irradiating them with various doses and then testing their ability to concentrate the vital stain, neutral red. It was found that irradiation, even with doses of several thousand rads, did not measurably affect the efficiency with which these cells concentrate the dye into their cytoplasm. Other investigators also have been impressed with the failure to find a nongenetic site in the mammalian cell that could explain the primary radiation damage (20).

While all of the data here presented do not exclude some as yet unknown physiological mechanism in the cell which is even more radiosensitive than the chromosomes, no definitive evidence as to the existence of such structures is as yet available. It is imperative to keep searching for as many different nuclear and extranuclear regions as possible, which may be involved in the action of radiation, either directly or indirectly. However, elucidation of the role of the chromosome damage itself opens up many important facets for study. While cell reproductive death is undoubtedly an essential, elementary process of the total mammalian radiation syndrome, it is certainly not the only one, and of relatively small importance for doses below the mean lethal range for individual cells. Cells in which gene and chromosomal damage have occurred to an extent insufficient to produce killing, may be far more important in the ultimate economy of the organism. Such cells, continuing to reproduce, would found lines of descendents within the body whose genetic constitution, and therefore, biochemical potentialities, have been permanently altered. Such metabolically abnormal cell lines might give rise to malignant disease at a later date, or could, by their disturbed biochemistry, contribute either to specific organ malfunction, or to nonspecific biochemical burdens which would add to the aging process. It is essential now to study in great detail the frequencies of the different possible types of chromosomal defects in mammalian cells, as a function of total dose, dose rate and metabolic state of the cells. It is necessary to determine how various biochemical pathways can be altered in surviving cells, and how such alteration may affect the subsequent history of reproducing and nonreproducing cells in the various body tissues.

Studies have also been carried out on the effects of

ultraviolet light on mammalian cells, and the ultraviolet-survival curve of the S3 cell was constructed (21, 22). It was of interest that, whereas this cell is much more sensitive to killing by X-irradiation than T2 bacteriophage, it proved to be less sensitive to ultraviolet. Originally we interpreted this finding to indicate that killing of S3 by ultraviolet may proceed mainly by the accumulation of single gene mutations. This explanation cannot be justified on the basis of the existing survival curves which could be explained by a variety of different mechanisms. However, gross chromosomal damage is demonstrable as a result of ultraviolet irradiation of mammalian cells. Assessment of the contributions to cell killing by the different actions of ultraviolet is now being undertaken.

The implications of these considerations for study of the effects of radiation on the aging process would appear obvious. Regardless of whether natural aging is or is not primarily the result of the accumulation of genetically altered cell clones within the body tissues, the production of genetically defective clones by irradiation would appear to contribute directly to general tissue malfunction. Since such radiation processes are largely random, the loss of fitness resulting from establishment of such defective clones would be distributed through many tissues. Since all the body organs are strongly interdependent, feedback mechanisms would cause impaired function from a small number of locations to impose stress widely throughout the body. It is interesting in this connection that we have found evidence that mammalian cells are more susceptible than bacterial cells to stress resulting from changes in the relative concentrations of nutrients present in the medium (23). This fact may reflect greater sensitivity of mammalian cells to biochemical imbalance.

The same kinds of studies as are current in the study of cellular action of radiation can now be carried on in the analysis of the aging process. Thus, sampling cells from animals of different age groups for in vitro growth, chromosomal investigation and, ultimately, genetic-biochemical analysis, should make possible determination of the extent to which the aging process is accompanied by genetic cellular changes.

Some new facts have already been secured. Thus measurement and identification of the human chromosomes revealed the much greater size of the X compared to the Y chromosome (24) and led to a possible explanation for the greater average longevity of the human female in terms of the greater amount of chromosomal material and, hence, biosynthetic factors of safety present in her cells. Now that certain well recognized human genetic defects like Mongolism, Turner's Syndrome and Klinefelter's Syndrome have been shown to result from extra or deficient chromosomes caused by nondisjunction, it becomes possible to calculate frequencies of chromosomal disproportionation in meiosis. The now well-documented occurrence of mosaicism in man should lead soon to estimates of mitotic nondisjunction. These frequencies should permit determination of the extent to which such processes may produce aberrant clonal cell lines. They may also lead to an understanding of the chemical environment that tends to induce these abnormalities in mammalian cells. Finally, as these methodologies are further refined so that it becomes possible to study definitively the action of large numbers of specific gene mutations, much greater understanding will be achieved about the mammalian cell genome and the effect thereon of time and agents like radiation.

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Genetic aspects of radiation injury and processes leading to normal senescence[1]

Genetics Panel Report

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A GROUP OF GENETICISTS gathered at Iowa State College on December 12–15, 1958, to examine the question, "Is there a relationship between normal aging and long-term radiation injury which may be defined in genetic terms or for which further genetic research would be desirable?" Participating in these discussions were: K. C. Atwood, J. H. D. Bryan, H. B. Chase, W. F. Dunning, Sterling Emerson, J. W. Gowen (Chairman), Douglas Grahn, H. L. Hamilton, H. O. Hartley, L. N. Hazel, Paul Henshaw, W. F. Hollander, G. H. Hunt, Oscar Kempthorne, J. L. Lush, H. J. Muller, P. A. Peterson, D. S. Robertson, G. A. Sacher, Janice Stadler, Leo Szilard, O. E. Tauber, Bruce Wallace, and Roger Williams. The primary objective was to focus attention on genetic factors, however remote, which may play a part in senescence, radiation effects on life span, or both, rather than to present a critique covering current knowledge available on this subject. Analytical evaluations may be expected at a later date. Background information is presented in the first three sections with integration of possible methods of causing the effect toward the end of the following summary of the entire discussion. Opportunity for comment and to make alterations of those sections that represent their own interpretations was provided for each speaker. Not everyone will agree on the relevance of all the investigations and suggested interpretations. It is hoped, however, that the material will assist in directing genetic thought to significant aspects of the aging problem. The following report is a summary of these discussions.

FACTORS ASSOCIATED WITH SPONTANEOUS SENESCENCE

Serious difficulties developed in defining "senescence." As Dr. Hunt said, ". . . with respect to aging, the problem is that no one has come up with a definition which is universally accepted. Some say aging is whatever happens with the passage of time. Others tend toward the pragmatic approach that aging is what happens leading toward death of a complex organism." Following either pathway can lead into some pretty devious byways. Yet, as Dr. Emerson pointed out, the investigator must specify the criterion used for evaluating the effects of the environmental or genetic stresses invoked in the senescence studies. It is worth while to examine some of the current information on senescence, if for no other reason than to demonstrate the incompleteness of the information and the need of further ideas for research. Discussion of the part radiation exposure may play in modifying these processes will be taken up later.

Senescence

Senescence means growing old—a retrogressive action: how an organism grows old. It also has a descriptive meaning, i.e., the characteristics that accompany growing old, such as graying and loss of hair, inability of the eyes to accommodate, and progressive decline in ability of the complex organism to handle its environment. But there is also the antonym, "juvenescence": the regaining or retaining of these lost attributes. In the cycle of self-duplication, egg to egg in higher forms, the sequences of cells in the germinal tract trace back through other ancestors eventually to single cells which, in turn, may go back to nucleic acids and associated proteins to carry what it takes for life continuity. In the interim, egg to egg, the soma is differentiated and set apart from the germ cells in a manner comparable with what also occurs in the single-celled organisms. The climax of senescence is death of the soma. Jennings (1) focused the problem when he said, "death did not take origin in consequence of organisms becoming multicellular." The changes are progressive and similar in each species, in each individual, although different in time. Maupus described them for Stylonychia 70 years ago. At first, the cells are turgid and actively dividing, metabolizing, and differentiating finely formed structures. With aging, measured from conjugation, cells reproducing by fission gradually become smaller. They take less food, do not assimilate well, and grow less well after each division. The appendages tend to disappear, the cell bodies to

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become distorted, and the micronuclei to become smaller, irregular, and vacuolate, then disappear. In some species, a strong tendency to conjugate may develop, even after degeneration becomes marked. Barred from some form of nuclear reorganization, all cells die. Rejuvenescence comes through reproduction associated with reorganization. Some cells in multicellular organisms may follow like patterns but have the added complexities associated with the delegation of particular vital processes to particular organs. The time intervals over which senescence acts vary enormously among different species. In nature, with its pyramid of parasitism, death often comes from chance accidents. In the laboratory, some of these accidents can be eliminated, and the operating biological forces, to some extent, may be revealed.

At fertilization, the cell is endowed with characteristics as well as potentialities for life. These characteristics differ tremendously among species. The initial cell for no species is totipotent for all living characteristics. Through evolution, there has been a progressive restriction as well as expansion of the possible characteristics carried by the gametes. In some instances these restrictions can be traced to genes. The development of the soma also displays restrictions as shown by Boveri, Driesch, and those who have expanded their work. Cells within the developing organism lose their capacity to produce the whole. This progressive mosaicism, although it differentiates organisms and makes them unique, makes the requirements for maintenance of their cells progressively greater. Failure in any item can cause death, or initiate progressive changes that are associated with senescence.

Senescence is a population problem as well as an individual problem. The population problem is described by life tables. These differ among cohorts within species as well as among species. The forces responsible for these changes become visible through analyses of the physiological and anatomical factors involved and the environmental and genetic causes leading to them. Similarly, the same forces are operating on the cells within an individual of a species.

INHERITANCE PATTERNS AND SENESCENCE

The inheritance patterns may be subdivided into four parts.

1. Cellular development, although prepared by genes, depends, at the same time, on the stage of preparation necessary for other genes in the genome to act. These factors are particularly evident when comparisons are made of life tables of different species or of individuals in a cohort within a strain. In *Drosophila*, there are genes that alter the egg cytoplasm before fertilization, so that any female progeny is destroyed, whereas the male progeny is unaffected (2). At fertilization, there are thousands of known genes that kill in the blastula or gastrula stages before the eggs hatch. In lethal (1) 7, melanotic tumors develop (3), and are accompanied by gut abnormalities that cause death in larval life at 3 or 4 days of age. Similarly, a gene that probably operates on the ring gland (4) results in failure of pupation and, although the larvae are of juvenile form, death by 20 days of age. Focal melanosis acts during pupation, expressing itself by focal melanotic degeneration of the leg joints, followed by death at 10 days. Tumors of the ovary express themselves later in the age sequence. The number of known genes necessary in the age sequence seems to be limited only by the study put upon them, i.e., in the silkworm, the genes operating on life processes are most frequent for the egg and larval stages and, in *Drosophila*, for those between the egg and early adult stages. In man, the onset of the different gene effects varies from early uterine to middle life; for example, Huntington's chorea generally does not become apparent until 35–50 years of age. Separation of late-acting gene syndromes has not been widespread in genetic work, because characteristics expressed early give more rapid results at reduced cost, but genes influencing these life stages are known, and many more are surely present. Genetic differences among individuals within a cohort give semblance to the mosaic of life expectancies observed in the life tables covering these groups.

2. Different genotypes operate with each other as well as interacting with environmental variables to increase the complexity of the senescence sequence. Strains of mice or domestic fowl react to different typhoid-like diseases in different ways: some show no or mild symptoms; some may become morbid but recover; and others may die. With a given pathogen and exposure, the genotype of the host may be shown to be a principal cause in this reaction, but if different genetic types of the pathogen are introduced, some will be highly virulent, some will have less virulence, and some may be nearly saprophytic. When this is done, a fourfold interaction takes place: a resistant host with a virulent pathogen may show no or slight morbidity; a resistant host with a medium-virulent or saprophytic pathogen may show no disease; a medium-resistant host with virulent pathogen may show more morbidity and die; and so on, until, with a susceptible host and a virulent pathogen, all hosts die. All forms of life curves may be generated by these interactions of the two genotypes (5). Comparable situations can occur with dosages of nonliving substances. The host-gene interactions with what is, broadly speaking, the environment are potent forces in the life-curve descriptions of senescence; sometimes the very diversity of these effects tends to make for other interpretations.

Graying of the hair or baldness in man or mice often occurs with aging. Yet, clearly, in mice and probably also in man, there are several genes that bring about graying or loss of hair at various stages in the life cycle. Some of the complexity of senescence is indicated by such cases. Something is known about the direct cause, and, consequently, it may be asserted that the changes do not portray the consequences of senescence since the causes are known (!). But it could be equally true, since so little is really known about the pathways of gene action, that

these cases are, in truth, those that, in their extreme effects, really show something of the paths through which senescence acts.

The preceding discussion has dealt with genotypic differences, among the organisms in a cohort, and their influence on life spans. Similar differences may exist among cells within individuals. These differences among cells may be either of a chromosomal or of a genetic nature. Under these circumstances, the individual becomes a mosaic of differing cell genotypes, on whose efficiencies and compatibilities will depend the life spans of the individuals involved.

3. Organisms may differ or their cells may be unlike in their chromosome complement. They may be haploid, diploid, or polyploid, and the differences appear as among tissues, within tissue within individuals, as well as among individuals. In these circumstances, the cells within individuals will ordinarily have like genes throughout, but they will differ from other individuals in gene dosage. More rarely, the gene complement may be hypoploid or hyperploid.

Not all polyploidy is unfavorable to life. In *Drosophila*, life tables of triploid females, having 3 sex chromosomes plus 3 sets of autosomes (3 X + 3A, 12 chromosomes in all), diploid females (XX + 2A, 8 chromosomes in all), males (XY + 2A), and intersexes (XXY + 3A) show that the balanced types (ratio of Xs:As = 1.0)—triploid and diploid females—have equal lives. The XY + 2A males, balance = ½, have somewhat shorter and the intersexes, = ⅔, have noticeably shorter life spans (6). Life tables covering radiation effects on these types follow a similar pattern. It is understandable that the lives of the males should be shorter. It is not so understandable why radiation should have about equal effects in life-shortening of the diploid and triploid females. The reactions brought about by the radiation must be complex and balancing, for any gene mutations occurring in the exposed triploid would seemingly do less damage, because of gene-dosage effects, than it would in diploids.

Similar complexity exists among gynandromorphic *Drosophila* and mice and for the haploid-diploid relationships observed in bees.

Life-span shortening is noticeable in *Drosophila* when the flies are variegated for particular cell types. The mosaics are created when chromosomes are broken, and the broken pieces translocate. The reorganized chromosomes pass through division of maturation and, hence, are transmissible from one generation to the next. They may display the dual nature of the cells throughout the body by the genetic characters which they carry. Four different mosaics, in which mosaicism may be demonstrated in such different organs as the eyes, wings, bristles, malpighian tubules, and testes, have been investigated for their effects on life span (7). The abnormal cell mixture within the types may be slight, in some individuals, or extreme, in others. These mosaics have the further property that the degree of mosaicism may be controlled by varying the genotype or an environmental condition. The presence of an extra Y chromosome reduces the phenotypic numbers of abnormal cells in the mosaic individuals to nearly zero. Low physiological temperature makes the mosaicism more extreme, whereas high temperature reduces it to near zero. When the proportion of abnormal to normal cells becomes large in flies of one type, subjected to low temperature during the larval period, the males all die, and the number of females is reduced nearly to half the number for normal controls. The surviving females appear debilitated. They are sterile in many cases and live but a short time. Reducing the amount of mottling through the addition of an extra Y chromosome or by growth at a higher temperature reverses the survival pattern. In flies, developed at a higher temperature throughout larval life, the ratio of abnormal to normal cells is greatly reduced; the males live and breed, and the females are essentially normal. Intermediate temperatures tend to yield intermediate ratios between abnormal and normal cells, and intermediate life spans for the different types. In these cases, it is evident that a mosaic of abnormal versus normal cells in an organism tends to hinder vital functions. When the condition is extreme, death may follow. Although the genetic events involved in these effects remain obscure, they are of particular significance to radiation effects on life span. The spermatozoa, creating the four types, were irradiated but once. The irradiation resulted in gene-chromosome changes that were transmitted to thousands of progeny. These changes, operating within the cells of the developing organisms, made each a mosaic of cells of different types. The degree to which each animal is mosaic influences its life span. These facts leave little doubt that somatic mutations, in the broadest sense, can influence the rate of aging.

Similar observations have been made by Hannah (8) in a study of the influence of the age of the maternal parent on the frequency and viability of the progeny types. Aging resulted in an increase in the number of gynanders, from 0.9% to 17.2%, after 11 days' aging. The number of female offspring was reduced. The change was linear and influenced by the genotype of the male parent. The progeny from males of the ring X chromosome genotype showed a much greater decrease of diploid females than when the males were rod X. Males with no Y chromosome were less viable than XY males, and aging the maternal parent decreased the incidence of such males. Gynanders and males with no Y chromosome showed poor viability during the first 24 hours.

4. Mosaic organisms may be formed from genetically different cell types. These types are mechanically held together, and must operate with each other to make or destroy the respective individuals. The heterogenicity may take place through a variety of genetic processes: gene mutation, somatic crossing over, mechanical transfer, implantation of tissues, and so forth. Most current knowledge on these conditions comes from a study of changes within germinal tissue.

The spontaneous, visible mutation-frequency for X chromosomes of *Drosophila* spermatozoa is about 6 ×

10^{-6} per selected locus per generation (9). The spontaneous lethal rate is variously determined as 0.1–1.3% for the X chromosome and possibly five times that for the genotype as a whole. Thus, 0.5–12.5% of all flies should carry new lethal mutations in each generation. Most matings must also transmit a certain number of mutations from the preceding generations. Accordingly, lethal factors in *Drosophila* are quite common.

From these data, it is tempting to conclude that, for any individual, the continual division and replacement of cells, which accompanies life, offers a mechanism for spontaneous mutations to create ever-expanding mosaics of the cells, as aging progresses. Since most of these mutations would be physiologically detrimental, a progressive decline in vigor should result. Such a decline is compatible with observed change in somatic aging of most species, and, to this extent, would furnish a model for that part of senescence which is visible in age-changes. Yet, to many experienced investigators of senescence, this is an unsuitable model. In many organisms senescence is attributed to cells, such as those of the brain, which, once established, divide but seldom or not at all.

The results of studies of the mutation frequencies of oocytes also caution against the ready acceptance of this view, although the differences observed after irradiation of males and females may be due to the physiological state of the chromosomes at the time of radiation exposure. *Drosophila* of several strains, differing in genetic constitutions and observed by different investigators, have, in some cases, exhibited spontaneous lethal mutations noticeably less often in the females than in the males. Spontaneous lethal mutations in 5,198 female gametes (10) showed a frequency of only 0.06%, whereas 4,860 tests for spontaneous mutations in males gave 0.62%. In 204 chromosomes of female origin in Florida-19, no lethal factors were observed by Glass (11), whereas in 717 chromosomes derived from males the spontaneous rate was 0.70%. In contrast, in some of our own data on 392 female chromosomes and on 286 chromosomes derived from males during the same investigations, the spontaneous mutation rate for lethal factors was zero. These observations pertained to *Drosophila* strains characterized by a relatively low spontaneous mutation rate. On the other hand, more extensive results with X-irradiation of the same strain support lower mutation rates for chromosomes derived from females. Should these sex differences also extend to the somatic cells and should aging be a function of the spontaneous mutations, the mean life spans of male *Drosophila* should be lower than those of the female, although possibly only slightly lower. As indicated by the studies reviewed earlier, this is the case.

Somatic Mutations

Somatic mutations are a direct means of altering the organism's genotype as it ages.

Bud mutations in plants often are preserved through cuttings. Some cuttings have been propagated for many generations. If the bud mutation is a part of the germinal tissue, some mutant cells may be incorporated into germ cells, which will later show the condition to be due to genetic inheritance. For many somatic mutations, the mutant cells alone are affected, the separation between mutant and normal tissue being sharp, cell for cell. Effects on the life span may come from the fact that the mutant cell is less efficient than the cell from which it originated, in the manner discussed earlier for the inherited, variegated types initiated by chromosomal translocations.

A type of mosaicism with less sharp limits occurs when stem cells of the circulatory organs become differentiated through cell transplantation, mutation, and so on. The cells resulting from divisions of the mosaic tissue pervade the whole organism and may influence many of its functions (12, 13). The time at which different cell genotypes are introduced into the soma may have profound effects. In cattle, reciprocal interchange of stem cells contributing to the blood-cell populations during fetal development of twins frequently establishes foreign blood types, which remain for rather long periods of the life span without apparent harm to either type of cell or the organism as a whole. Organisms are more ready to accept foreign antigens, and so forth, to their benefit at certain ages or in certain environments, i.e., before or at birth or after irradiation, than they are in other circumstances. Mixed phenotypes, generated by somatic mutations, would presumably behave in like manner. Yet, similar mutation mosaic in later life may be suspect. Skin transplantations, blood and bone marrow transfusions, and other comparable procedures, frequently elicit the operation of histoincompatible genes that are so highly incompatible with the new types as to suggest that cells derived through mutation within an individual could lower biological efficiency and contribute to senescence.

Somatic mutations of another type may influence every body cell. Circulating hormones that have access to the body as a whole have frequently been shown to be under genic control. Mutation could cause the resulting cells to cease, limit, or increase secretion. Thus, a vermilion genotype in *Drosophila*, in which part of the cells are wild type will express only the wild type somatic characters of brick-red eye color and yellow testis. The wild type cells of the mutant form kynurenin, which is made available to the rest of the organism to complete the synthesis of the eye pigments.

Research on the quantitative aspects of somatic mutations in animals is still in its infancy. Present data allow only inferences, drawn from studies of germinal cells, about the rates and kinds of mutations in somatic cells. Goudie's earlier (14) and Atwood's current investigations on human blood-group antigens were designed to collect data that would allow estimation of these rates. Examination of the cells in the peripheral blood has several advantages: there are adequate supplies of cells and distinct phenotypic effects for both original and mutated genes, and possibilities of repeated tests on the

same individuals. Moreover, mutations in the stem cells of the hematopoietic system are multiplied manifold. The difficulties are those inherent in agglutination reactions, which require tedious techniques for quantitative work, but a limited beginning has been made to assess the possibilities that somatic mutations offer in explaining the changes observed in senescence.

Developmental Sequence

The sequential development in life patterns, although traceable to genes, also stems from a need for certain products of gene action to precede those organized by other genes in later stages of development. Spermatozoa or oocytes can carry genes lethal to the differentiated organism as a whole, but not lethal to the gamete itself. The egg can sometimes develop through many cell divisions (until the larva is fully formed) before a lethal condition, i.e., focal melanosis, develops. Similar sensitizations in specific locations in but a tiny part of the body are often necessary for given normal genes to show their potentialities, i.e., the speck gene in *Drosophila*. The surrounding cells also have the gene, but conditions are not suitable for its expression.

These events often occur early in life, but are not limited to that period. They may be more prominent in later life, blended with what appear to the observer to be the sequential changes characteristic of senescence. Dr. Dunning, in reviewing pertinent observations collected in the 40 years of continuous research in her laboratory, much of it conducted with Drs. Curtis and Bullock, cited some results that, in this connection, are significant. Thymic tumors in Copenhagen rats are specialized tumors of high incidence in the latter third of the life span in this rather long-lived strain. Other strains do not show these tumors. They are an extreme example of inherited characters, known to be present in the fertilized egg, but acting only in the latter third of the life span.

Inheritance patterns of life processes furnished by these rats give further evidence for the inherited discontinuity of many of the processes frequently listed as factors in the aging process. These investigators isolated several inbred lines of rats, differing in several particulars. Selections were not made primarily for longer or shorter life spans, but the resulting inbred rats did differ in life spans by as much as 80%, based on averages from some thousands of completed rat lifetimes. The short-lived strain differed from the long-lived in other intangibles that may be associated with rapid senescence, as contrasted with slow senescence. Crosses of these strains established genetic bases for these differences. The hybrid progeny from the short-lived strain by long-lived approached the life spans of the long-lived parents; they also had many of their less readily measurable characteristics. Some maternal effect was carried over to the progeny, affecting even this long-term character. Backcrossing of the F_1 to the long-lived parents gave long-lived progeny. Those to the short-lived inbred resulted in progeny with but slightly longer life spans than the short-lived parents. Results like these have come from a limited number of recent experiments conducted by other workers. They include a wide range of species from microorganisms to other species of mammals.

The results also include other factors that are correlated with accelerated or retarded aging. Rats of the slowly aging strain have more polymorphonuclear leukocytes, which may be significant in the general resistance to disease; they explore more, have greater emotional stability, and hoard less; and there is a real difference in sitting time.

Extensive studies were made of the appearance and characteristics of a large series of tumors that normally occur late in life and are associated with senescence. These tumors resulted from invasion by a living agent, *Cysticercus fasciolaris*, and occurred in middle to late life; the low incidence in the short-lived strain was owing to that cause. Infection must occur early in life, and it takes about 9 months for establishment of tumors. Infectious dosages and total-cell contacts with the initiating agent are important. Clearly, the low incidence of tumors in the short-lived strain reflected merely the lack of time for development.

In the same manner, genes that find expression late in development may not be lethal, whereas they might have been lethal shortly after fertilization. Genes are known that are lethal to the organism as a whole, but that, when segregated in late development, are not lethal to cells in which they are then contained (15). Such cells are either supported by substances contributed by the surrounding normal cells to make up the deficiencies, or the development through which the organism has passed is beyond the stage of lethal action.

Phenocopies offer another means by which specific gene actions may be simulated and affect vitality. The characteristic (16) of this process is that the effect is visible only within the individual, and ends with the sexual phase. The decisive factors are agents more powerful than those normally present, treatment at a critical period of development, and suitable genotypic material. The phenocopies then give reproducible properties to the somatic cell lines. The action of some carcinogens may be of this type. There is evidence that the genotype contributes to the host the likelihood of development of special tumors. The tumors are frequently lethal in the hosts but are not transmissible in sexual inheritance. They may be cultured in vitro or be transplanted into similar hosts.

These considerations are closely allied to those involved in *Dauermodifikation*, alternative steady-state conditions, self-perpetuating adaptive enzyme accentuation, and modification or even reversal of gene action.

Somatic Crossing Over

Yet another type of somatic mosaic, which has been made visible in *Drosophila* by suitable genes but not yet proven for mammals, is dependent on the occurrence of somatic crossing over (17, 18). If somatic crossing over is

a general process and not dependent on the somatic chromosome-pairing observed in Diptera, it could be important in the senescence of all animals. Mosaics owing to somatic crossing over occur spontaneously, but the frequency of occurrence may be augmented by the presence of particular genes in the host genotype. In Stern's data, mosaics were detected by using the sex-linked genes, yellow and singed. The visible mosaic cells were those resulting from crossing over in the sex chromosomes. Data about the autosomes and other gene loci could considerably increase the frequency of detectable somatic crossing over. Of 1,322 flies examined, 141 (about 11%) were distinguishable mosaics. In the presence of the minute genes, 1,019 flies showed 344 spots; 33% were known to be cell mosaics. The size of the mosaic spots was generally 1 to 4 setae; only rarely was it 50 or more. The area of the mosaic indicates the time when, in the sequence of somatic cell divisions, the somatic crossing over, which led to the formation of the organism, occurred. Somatic crossing over occurred most frequently in the late cell divisions. Since individuals within most species are highly heterozygous, somatic crossing over could furnish a means by which each individual within the species may become more highly mosaic with age. A large proportion, indeed, perhaps all individuals should be mosaic for different types of cells. If such a mosaic condition were detrimental to the host's best functioning, the life span would be adversely affected. If the original diploid egg cell is heterozygous for a lethal factor or a deficiency, one of the cell products of somatic crossing over will become homozygous, and its descendants may be less vigorous and even exert a detrimental influence on the normal cells that make up most of the organism. For lethal genes found for loci covering 15 regions occupied by genes having visible effects—w, fa, ct, t-amx, m, wy-s, g-ty, Mof, f, B^+, Bx, M, Mn, y-ac, and dy—only two y and ct, were not lethal to the cell when homozygous. (19). Some were believed to include small deficiencies. On the other hand, of 24 lethal genes not known to be allelic to any known loci causing visible phenotypes, 14 were found to be viable in hypodermal cells of the female. The cell deaths established somatic crossing over as a mechanism that can result in lesions and the death of cells throughout the body.

Somatic crossing over opens further possibilities of mosaicism. Yeast genetics has indicated that gene duplication may not be exact, as once thought. Occasionally, instead of the 2:2 ratio of gene products expected in meiotic division, 3:1 or even 4:0 ratios may occur. To account for these ratios, it has been suggested that one of the genes of the allelic pair influences the duplication of the other so that it duplicates twice or oftener, whereas the other does not. If this process of gene "conversion" carries over to somatic cells, further diversity in gene material is created on which somatic crossing over may operate to increase phenotypic mosaics.

The foregoing information covers descriptions of a number of genetic processes by which individuals might become mosaics of two or more cell types, which, through their individual requirements, could be detrimental to life and lead to senescence.

CYTOPLASMIC-NUCLEAR INTERACTION

The significance of cytoplasmic-nuclear relations to the aging of cells within clones of certain microorganisms was discussed by Dr. Emerson. In *Neurospora* and in *Aspergillus* (20), aging may be measured by the length of time a strain can be maintained vegetatively or by its reproductive capacity. Reproduction may take place through ascospores, conidia, or hyphal tips. If reproduction for several generations occurs by conidia-formation, there is a gradual loss of capacity to form perithecia and ascospores. If the reproduction occurs by hyphal tips, there is also a loss of perithecia and, later, a loss of conidia. In reproduction through ascospores, no loss of either of these attributes occurs. The loss of function may be completely reversed by one ascospore generation. At any stage in the degeneration, while perithecia-formation and ascospore-formation yet remain, if reproduction through ascospores is accomplished, there is full return to the normal sexual potentialities of the cell descendants. It was similarly found that the deteriorative effects of the aging were transmitted through hyphal tips or conidia, but not through heterokaryons. Heterokaryons behave as though they were young cultures. As has sometimes been observed in protozoa, the results suggest the transmissibility of degenerative changes through cytoplasmic elements, rather than by nuclear materials. Cytoplasmic transmission is further suggested by observations on mercuric chloride tolerance. The tolerance is transmitted in reproduction by hyphal tips or, to a somewhat lesser degree, by conidia but not at all through the sexual fusions involved in ascospore-formation.

RADIATION EXPOSURE AND SENESCENCE

Heavily irradiated animals or plants receiving acute dosages of 1,000 or more times the yearly rate of background radiation or repeated irradiation often react as though the radiation had simply acted to accelerate the natural processes that are presumed to lead to senescence. This mimicry has been noted in individuals, and is seen in changes in the average life spans observed in life tables. The question is: do common causes exist to produce the like effects or is the similarity simply superficial and spurious?

The factors transmitted through the germ cells, as discussed earlier, account for much of the variability in the expression and duration of senescence, although the mechanisms by which these changes are physiologically produced are by no means clear. However, primary radiation effects, those acting directly on the individual, are, presumably, effective in tissue cells other than the germ cells. Accordingly, the radiation effects must operate in tissues in which cells are at least diploid and sometimes polyploid, as in a significant proportion of

the cells of the mammalian liver. In consequence, the direct effects of such mutants are reduced owing to normal alleles, which balance the effects of the mutant. Similarly, for any one genetic change, the mutated gene would probably be found initially in only one cell of a tissue. This cell could replicate to yield more like itself, but the tissue would always have a reserve of normal cells to compensate for the effects of those that are abnormal through mutation. Ablation experiments on organs or parts of organs show that the degree of compensation can be large. Similar considerations show that somatic radiation gene mutations would be handicapped as contrasted with gene mutations in germ cells, in increasing the rates of deterioration necessary for senescence.

Dr. Atwood's observations on blood group antigens are a case in point. The genes responsible for the blood group antigens, should they mutate, can be identified in the heterozygous condition. Since it is the reproducing stem cells which mutate, these cells will multiply the mutations at each division and make the individuals mosaics of different types. Although the evidence obtained on cattle twins, but not collected for this purpose, indicated that some individuals which are blood group mosaics are not handicapped in vigor at early ages, more data concerning more genes are needed to establish any pattern. Cell variegation in *Drosophila* (7), brought about by other causes, is certainly detrimental to the proper functioning of the individual and its life span.

There are occasions when the detrimental somatic mutations may occur in, or be multiplied to, greater numbers than at others. In early development there is a progressive branching off of specialized cells, which become differentiated organs. The total volume of the chromosomes of these cells is several times that of the fertilized egg. The points at which radiation damage may occur become numerous. Since development consists of multiplying the few vital cells to many, the total damage to the final organ soon becomes serious to the individual. Experimental evidence indicates that the damage from irradiating embryonically developing organs is disproportionately large, and, when studied on an age basis, appears to take on a wave form with peaks at different phases of development. These peaks correspond to actively developing phases of different organs.

Each instance of irradiation leading to somatic changes that are regarded as conventional signs of aging raises the further problem of determining whether the effects are owing to genes or owing to changes in the matured-cell cytoplasm, which is no longer under control of the genes. This dilemma was brought into focus by Dr. Chase. Graying of hair and epilation, hyperkeratinization, and hyperpigmentation of the skin are all recognized signs of aging. In mice, all of these conditions may develop in response to genes transmitted through germ cells. The time in development at which the conditions appear is variable with the particular gene and, to some extent, with the environment. Since the somatic cells develop from the germ cells, gene loci are present in the somatic cells and could mutate, under irradiations, to cause graying of the normal mouse owing, to dominant or, when homozygous, recessive mutants.

Somatic coat changes show dose dependence to irradiation, just as they show a recognized age dependence under normal spontaneous exposure, which has led to including them among the senescence phenomena. With from 300 to 1,000 r, graying occurs readily, the low dose being necessary for the resting follicle. Temporary epilation follows doses of 400 r to the young follicle, and 1,400 r to the resting follicle. Exposure to 2,000 r leads to ulceration; to 3,000 r, to hyperpigmentation; to 4,000 r, to permanent epilation; and, for some mice, to 3,000 r, to tumors of the skin. The graying of the hair is owing to loss of stem cells for pigment formation. In aging, the graying is generalized. By irradiation, it can be made highly localized and dependent on the stage in the growth cycle of the follicle at the time of irradiation. When these factors were controlled, mice heterozygous for some of the coat-pigment genes were no more prone to graying because of irradiation than those that were homozygous. This fact indicates that the mechanism for normal graying is not one of cell mutation or chromosome elimination, but is of some other type.

Studies of the areas that had undergone irradiation but were examined about a year and a half later had some rather surprising results. Since hyperpigmentation in the mouse occurs with increased age, it seemed possible that the areas of hyperpigmentation owing to irradiation might have faded somewhat in the interval between irradiation and subsequent examination, but might age at a greater rate than the unirradiated areas of the coat. No evidence was found for a change in this direction. In fact the control areas of the skin looked older than those which had been treated. These observations make it difficult to regard irradiation as a senescence phenomena. However, another factor may be involved. There is a rejuvenating of the mouse skin as related to hair-growth cycles. Aging in the control areas may be associated simply with lack of these cycles, whereas, in the irradiated areas, the irradiation results in the slipping off of the old cells with a replacement by those of younger age and greater vigor.

SOMATIC RECESSIVE GENE MUTATION

Recessive mutations must have some effect on the phenotype in heterozygous state or some mechanisms be available for making them homozygous at some later stage in development, if they are to exert a significant influence on senescence.

Truly recessive lethal or detrimental genes in the heterozygous state, by definition, show no effect on the survival of the individuals carrying them. Practically, there may be some effect either of heterozygosity or of reduced survival. Stern *et al.* (21) studied sex-linked, recessive, lethal genes for their heterozygous effects on vitality in individuals some generations removed from the first occurrence of the lethal factor. Similarly Umaerus and Gowen (unpublished observations) have

searched for signs of reduced vigor in different heterozygous lethal genes in the generation of individuals immediately following the appearance of the lethal gene. In both studies the average effect of the heterozygous lethal was a slight average depression of the numbers of individuals which were carrying the lethals, with a lesser depression of those which were carrying semi-lethals. The depression was of the order of 2–4%. The curve for the viabilities of the flies bearing the different new lethals was unimodal, with a rather narrow major range—between 40 and 57.5% survival (50% expected)—but with long tails on either side of this point, suggesting that the individual lethals may vary considerably in their effects on viability or in generating heterosis. Muller (22) and Morton, Crow, and Miller (23) studied third chromosome lethal-carrying heterozygotes and observed similar effects.

The evidence for production of lethal or detrimental recessive genes in the soma is rather small, so the argument that the recessive lethal genes in themselves, in the heterozygous state, cause the observed declining vigor of senescence largely depends on observations of the germ-cell tract and the expected parallelism of these results with those of cells in the soma. This interpretation is made somewhat insecure by the observations of Wallace (24) that *Drosophila* may accumulate up to some 90% of recessive lethal mutations in one of their chromosomes and yet have the population reproducing at an adequate rate and sufficiently pliable for maintenance. Such evidence also tends to rule out the possibility that two or more recessive heterozygous detrimentals, operating in separate loci, produce the detrimental effects of senescence through interaction. The interpretation entails further difficulties in that tests by Wallace (25) of sets of irradiated chromosomes versus their previous unirradiated homozygous homologues (wherein the irradiated chromosomes might be expected to contain mutated genes detrimental to survival) gave no evidence of superiority of the unirradiated chromosome.

To be most effective in severely reducing average vigor through their effects on the soma of a cell mosaic, it would appear that any recessive gene mutations occurring either in spontaneous senescence or as a result of radiation must become homozygous. At least two such mechanisms, partial or complete chromosome elimination and somatic crossing over, offer this possibility.

SOMATIC DOMINANT GENE MUTATIONS

Dominant gene mutations occurring in somatic cells offer immediate possibilities of influencing life span and loss of vigor. As they occur they create a mosaic of cells within the soma. If the dominant mutations are somatic lethals, only those cells in which they occur would show the effects, whereas the other surrounding tissue cells would be normal. The death of cells, so affected, would result. The necrosis of one cell, in most instances, probably would not be an important factor in the welfare of the organism, but occasionally—should it be near a capillary or in an organ secreting vital materials—it could materially affect other cells in depriving them of suitable circulation and the like, with the consequence that the necrotic area could spread to a point at which it would become detrimental and could be observed as an infarct.

Both dominant and recessive mutations are known to affect circulating products of several of the secretory glands. If such somatic mutations were to occur in cells whose viability is not impaired, but which could multiply and form tissue mosaics, they could, in turn, influence the viability of the whole organism through the secretions which they generate. Such genes are known to occur in the germ-cell tracts, and to be transmitted, thereby, to the soma. Thus, mechanisms are available that could accomplish senescence through somatic mutations. However, extended research will be required to establish that these mechanisms really operate on the somatic cells or that the mutation rates in the soma are sufficiently high during the life cycle to cause appreciable senescence.

SOMATIC GENE INTERACTION

Senescence in individuals as members of a population invokes forces additional to those involved in senescence within individuals. The population may be a mosaic of individuals that show various differences in resistance to infectious or degenerative diseases. In mice, the severity of typhoid largely depends on the particular genotypes of the mice exposed. In a heterogeneous population, all types of life curves may be obtained, depending upon the particular mixture of mouse genotypes that may compose the initial population. But the severity and outcome of any attack of mouse typhoid is also due to the genotypes for invasive power carried by the pathogens found in the population. These genotypes range from those that make it possible for the pathogen to live almost saprophytically to those that cause mild disease and, on, to those that cause death. Again, the life curves for a population of mice may take any form, dependent on the type of pathogen entering the population. For diseases in general, senescence may either increase or decrease resistance The radiation studies of Stadler (26) show that, for *Salmonella typhimurium* infection, natural resistance was decreased by rather large quantities of X-irradiation given to the host animals. The decreases were common to the six different genotypes of mice used in the experiments. Partial body irradiations to any of three regions—head, middle, or rear—also led to decreases in resistance, although the summed total effects were by no means as great as those for mice receiving whole body irradiation. These results indicate that the parallelism of decreasing resistance with senescence and with radiation are not strictly comparable. They suggest that other forces may be acting. They also indicate that any cells within the body may play a part in the resistance to this infectious disease.

Active acquired resistance, on the other hand, was not appreciably reduced in those mice which survived the

attacks of the initial disease. Acquired resistance was not appreciably affected by irradiation even though the original genotypes of the mice ranged from extreme susceptibility to extreme resistance to the first contact with the organism.

Dr. Sacher considered the manner in which these various problems affect the analysis of life tables. He pointed out that, basically, aging consists in all those changes in organisms that lead to an increased probability of failure of function which may be defined as mortality. The logarithm of the mortality rate is the most revealing statistic. In any species, the mortality rate increases by a factor that is roughly constant at all ages. Superimposed on this pattern may be mortality introduced by particular diseases or environmental factors of limited duration. In irradiated organisms, reduction in survival is roughly proportional to a combination of the first and second power of the radiation dosages. Radiation exposure of animals leads to an increase in death rate that is roughly characteristic of an older group of animals, after the acute mortality immediately following high radiations has been passed. Superimposed disease or other external conditions may affect the survivorships to further increase the mortality rate.

Changes in the observed logarithmic rates of mortality in animals which had high genetic susceptibility to amyloid disease were discussed by Dr. Grahn. These diseases are manifest in the life curves as sharp breaks at the time of onset, when mortality rises logarithmically. Other diseases may add other forms of curves that affect the basic trends but are, in a sense, superimposed on them and may be corrected for in the analysis of normal senescence and radiation aging effects.

A hypothetical model, in which one assumes as basic to aging an "aging hit" that "destroys" a chromosome of the somatic cell, was considered by Dr. Leo Szilard (27). The developed theory, including other assumptions, has been fitted to the U. S. female life curve for humans after age 40 years. Difficulties appeared in its application to earlier ages or to U. S. males. Particular interest attaches to this approach since, if irradiation increases these "aging hits" in the assumed manner, the aging (death) rate would then be predictable. The validity of this model remains to be established.

CHROMOSOME BREAKAGE

Chromosome breakage, as the basis of the somatic damage produced in irradiated individuals, was particularly considered by Dr. Muller.

The seeming possibility of fitting to a straight line the relation between accumulated somatic damage and the total radiation dose, when the latter is delivered at low rates, suggests that such damage reflects individual hits on the chromosomes. Induced malignant lesions, such as the leukemias in man studied by Lewis (28) and the mammary tumors in rats studied by Bond et al. (29), could plausibly be interpreted as the results of genetic mutations, whereas aging effects—i.e., the hindering of growth and regeneration; necrosis of tissues, including the regression of tumors; as well as much short-term somatic damage—would more readily be interpreted as being occasioned by chromosome breakage.

There are two main processes by which single chromosome breakages, induced by irradiation at low dosage, can damage somatic cells or their descendants. The main cause of death of early *Drosophila* zygotes derived from irradiated sperm (30, 31) is through the formation of chromosome bridges by the dicentric isochromatids resulting from union of centric sister-chromatid fragments. Here the conjoined nuclei later give rise to polypolar figures and giant cells. This process might equally well occur in dividing, irradiated somatic cells.

An alternative process takes place when the dicentrics fail to form a permanent bridge. The daughter nuclei succeed in isolating themselves, but parts of the broken chromosome are left outside the newly formed nuclei and degenerate, resulting in hypoploid cells that lack one chromosome. (In some cases, induced nondisjunction, not caused by breakage, also gives hypoploids, and a smaller number of hyperploids.) Hypoploidy may, in some cases, be fatal to the cell (as is the case in young *Drosophila* zygotes when a major autosome or the one X chromosome of the male is involved), and certainly impairs the functions of survivors. Such impairment commonly results, in diploid animals, from hypoploidy in itself, but it would be increased in cases in which the remaining member of the affected pair of chromosomes has inherited (or has had induced in it) a cell-damaging genetic mutation.

Recent experiments by Oster (32) at the University of Indiana, show that, after irradiation of immediately pre-pupal larvae of *Drosophila* with 3,500 r, the rate of survival to adulthood is much higher for ordinary females than for ordinary males; but the survival rate is very much higher for ordinary males than for males with ring X chromosomes. These results clearly point to chromosome loss by breakage as the chief cause of the mortality. That death is caused by hypoploidy and not by bridge-formation in this case is shown not only by the sex difference (inasmuch as a Y in the male may be expected to break and form bridges about as often as an X in either male or female), but also by the further finding that after such treatment, females with a ring and an ordinary X have about as high a rate of survival as do females with two ordinary X's. Further positive results in tests of this matter on *Drosophila* have been obtained by Muller (33). These include measurements of survival of females with attached X's, which should be about as much below ordinary males as males are below ordinary females; experiments with flies heterozygous for deficiencies and for diverse lethal factors; and experiments involving ring Y chromosomes. The tests should also be extended to treatments of other stages and determinations of the longer-term mortality rates. Whether spontaneous loss of chromosomes contributed significantly to natural mortality and aging in *Drosophila* can

be established only by large-scale, parallel comparisons among genotypes of the various kinds above mentioned.

It would be desirable to obtain similar data regarding rates of mortality and aging in irradiated and unirradiated vertebrates of critically different genotypes. Sex differences offer one approach for such experimentation. The waltzing deficiency found by Gates in mice, but now lost, showed that suitably deficient chromosomes induced by irradiation could be obtained in viable stocks. Possibly some types of whole-chromosome hypoploids could also survive in vertebrates, for example, in Amphibia. It would be worth attempting to learn whether haploid individuals could survive when aided by diploid parabionts, since haploid individuals would provide especially critical evidence on the effects of chromosome losses. Amphibia would also provide useful polyploid situations. This would give evidence of whether the damage caused by induced chromosome breakage is here a consequence of bridge-formation or of hypoploidy. For the damage caused by bridges, reckoned in terms of cells killed, would be directly proportional to the degree of ploidy, whereas that caused by hypoploidy would be greatly diminished by increased ploidy. In addition to comparisons between damage to entire individuals of different degrees of euploidy and aneuploidy, similar comparisons should also be made of tissues or parts of tissues (e.g., tetraploid versus diploid samples of the liver or of tumors). Tissues in culture lend themselves to such studies, as do grafts.

Among the questions requiring investigation is that of the relative kinds and degrees of somatic damage—including aging (whether induced or spontaneous)—caused by the actual killing of cells as a result of bridges, hypoploidy, or both, as compared with the somatic damage caused by cell impairment short of killing. It seems probable that, in tissues whose cells readily undergo regenerative mitoses, the outright death of cells, if it does not affect too many cells at once, might be almost wholly compensated for, whereas permanent (genetically based) cell impairments would in many cases have more serious effects since, simply by remaining alive, such cells would tend not to be replaced, yet would not adequately fulfill their functions.

As for the "noble" cells (such as those of the brain and some other vital organs) which remain practically without further mitoses throughout adult life, these could be expected not to be directly damaged in functions to a significant degree or in a permanent way by radiation or by other mutagenic agents. However, they could be damaged indirectly, through damage brought about in some of the cells capable of mitosis, such as the connective tissue, blood, blood-vessel-lining, and epithelial cells that serve these "noble" cells.

Attention should be drawn to the bearing of studies on individuals and tissues with different types and degrees of ploidy, or with deficiencies that forbid some types of hypoploidy, on the problems of the basis of induced and "spontaneous" malignancies. It is evident that, if they are caused by more or less recessive genetic mutations (whether singly or in combinations), they will be more readily and extremely expressed in haploid or hypoploid than in diploid, and much less so in polyploid, individuals or tissues. Moreover, their frequency will vary with a higher power of the dose wherever the cells are more polyploid. On the other hand, if such malignancies are caused by losses of chromosomes, they would not be expressed in haploidy at all, since the affected cells would die off. Fortunately, chromosome studies can be made in tumors, because of the numerous mitoses, so that this alternative can sometimes be checked by direct observation.

VARIABILITY OF RANDOM AND INBRED POPULATIONS UNDER IRRADIATION

Random-bred and inbred strains of different populations often show wide differences in life span within the different cohorts. Similar differences frequently extend to the sizes of different organs and to their physiological functions, as Dr. Williams pointed out. The degrees of intercorrelations of these variations and their influences on life spans are still moot problems. The variations are significant, but, in most instances, we can only speculate on the causes. The variations may be generated by the various genetic influences previously discussed or by environmental factors.

IRRADIATION AND PHENOCOPIES

As indicated earlier, under natural conditions, phenocopies occur which duplicate the effects of many mutant genes having more or less detrimental action on life. The parallelism between changes of life span induced by irradiation and those brought on by the natural senescence processes is further emphasized by the fact that irradiation increases the incidence of these phenocopies. Insofar as the phenotypes are detrimental to the somatic cell dynamics they would be contributing causes to senescence phenomena. In higher animals, these effects require much more extensive investigation. The production of adaptive enzymes—which are, in a sense, phenocopies of other genetic effects—in fungi and bacteria is of significance in this regard. These effects are interesting in that they may be lost through temperature changes, by holding the organisms without cell division for some time, and in the processes of the nuclear breakdown accompanying meiosis. Against the view that phenocopies are significant to aging is the test in *Neurospora* described by Dr. Emerson in which all of the radiation damage to heterokaryons could be explained as direct damage to the nucleus.

SOMATIC CROSSING OVER

As indicated earlier, somatic crossing over furnishes a mechanism by which recessive mutations may become homozygous in some cells of the body and, in consequence, have opportunity to express their effects. Should these effects be lethal, at different stages in development, they will create mosaics of necrotic cells, throughout the body of the animal, that may materially

affect the vitality of the organism. The parallel between natural senescence and radiation as a factor in life-shortening is further emphasized by the fact that irradiation in *Drosophila* materially increases somatic crossing over as shown by the results of LeFevre (34). Exposures to 1,300 r caused somatic crossing over to increase by 50–100-fold. *Drosophila*, however, by reason of somatic chromosome pairing, offers a special opportunity for somatic crossing over; it is not known whether somatic crossing over occurs to any significant degree in vertebrates.

CONCLUSION

Some genetic aspects of senescence and the effects that irradiation may have in accelerating life-shortening have been considered. By no means all the genetic possibilities in these interactions have been discussed. Others are known and, no doubt, new ones will be discovered. The data are sufficient to indicate that much more research on the problem, utilizing the genetic approach, will be profitable and lead to a better understanding of the effects involved. Several known phenomena, having a genetic basis, may be significant to senescence. Mechanisms worthy of current consideration include somatic mutations, genic interactions, chromosomal breakages and losses, phenocopies, and somatic crossing over. Since the methods discussed have appeared fairly recently, it may be expected that, as time goes on, those already discovered will be clarified, and others become known.

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Summary

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REVIEW OF THE REPORTS from the various panels leaves one with the impression that the concept that radiation elicits premature aging is a half truth which largely reflects the crudity of the original parameter–life expectancy.

There is no doubt that the post-irradiated animal bears damage in the genetic apparatus of its cells. This must surely diminish the reserve of the stem lines of the constantly proliferating tissues and hence enhance susceptibility to a second dose of radiation. Moreover many of the parenchymatous cells of such an animal must also carry damaged genetic components, but the extent to which these influence the life of those cells is not calculable at the present time. The significance of such damage in a nondividing cell cannot yet be evaluated and it seems unlikely that this will be possible until further information is at hand concerning the role of the nucleus in protein biosynthesis in the mature cell.

No data are at hand to contradict the concept that normal senescence reflects the loss of cells in post mitotic nondividing tissues. Highly suggestive evidence indicates that such cell death may be occasioned by alterations in the connective tissue particularly of the capillaries although numerous possible mechanisms for cell death intrinsic in the very nature of the cell itself warrant serious consideration. To the extent that various studies have provided a picture of the time-dependent deterioration of the capillaries of postirradiated animals which can readily account for the gradual death of nondividing postmitotic cells in all tissues of the body and hence diffuse deterioration of virtually all tissues so that the immediate causes of death are as varied as they are in the nonirradiated aging animal, the delayed effects of radiation and normal senescence may appear to be equivalent. Although not quite so clearly demonstrated, there is reason to believe that the connective tissue barrier between parenchymatous cells and their surroundings may well undergo a time-dependent deterioration with results similar to those observed in the irradiated animal. Were this hypothesis correct then indeed would it be true that mortality is a built-in penalty for multicellular existence.

Indeed the only common factor which has clearly emerged to date is the arteriolocapillary fibrosis which develops in the postirradiated animal and the related changes which appear to occur in the vasculature of a normally aging individual. The complexity of mammalian existence intrinsic to the very organization of a metazoan creature and the diversity of stresses which the environment may impose upon the total organism should certainly result in the great variety of apparent causes of death in the two situations. This does not deny that other underlying causes for the normal aging process exist in the mammal all of which must also be operative in the postirradiated animal which, in addition, suffers in a greater or lesser degree from chromosomal damage to somatic cells. In this sense the original analogy between the two situations is warranted but whether the resemblances or the differences are to be emphasized is a matter of personal choice at the present time. Clearly neither process is sufficiently understood